Behavioural drivers of fertility in red junglefowl *Gallus gallus* and commercial chicken flocks

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To my beloved Omi,

Gisela Pasternack

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"… a cock called Chanticleer.
In all the land, at crowing he'd no peer;
…
His comb, redder than the finest coral,
Was crenellated like the castle wall.
His bill was black, and as the jet it shone;
Like azure were his legs and toes, as one;
His claws were whiter than the lily flower,
And like the burnished gold all his colour.
This noble cock had in his governance
Seven hens, his pleasure to advance,
Who were his sisters and his paramours,
And wonderfully like him, as to colours;
Of which the fairest, tinted round her throat,
Was called the lovely lady Pertelote.
Courteous she was, discreet and debonair,
Companionable, and bore herself so fair
From the day that she was seven nights old,
That truly the heart she held in her hold
Of Chanticleer, locked in her every limb.
He loved her so that all was well with him.
And such a joy was it to hear them sing
When that the bright sun began to spring,
In sweet accord, 'My love's in foreign land'.
– For at that time, I'm given to understand,
Beasts and birds as well could speak and sing."

- Chaucer, *The Nun's Priest's Tale*, from *The Canterbury Tales* (1400), translated by A. S. Kline

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Abstract

The fowl, *Gallus gallus*, has been used to study sexual behaviour and sexual selection for many years: its combination of reproductive biology that is fairly typical of birds and a polygynandrous mating system makes it an ideal model species for studying the effects of pre- and post- copulatory competition and of conflict between the sexes. There is a large body of research spanning many decades on these processes in domestic chickens and their wild ancestor, the red junglefowl. As well as being an ideal model system, the sexual behaviour and fertility of this species is of great significance to the poultry industry, where the reproductive efficiency of broiler breeders (birds whose commercial purpose is to produce hatching eggs) is a key concern. However, the behaviour of broiler breeders in a commercial setting, and how their behaviour links to fertility, is little understood.

Females of this species are subject to intense sexual harassment, and some behavioural strategies exhibited by females in response to sexual harassment have been described. However, we lack a detailed and systematic understanding of the suite of female responses to sexual harassment, despite these having the potential to significantly affect reproductive success for males and females.

In this thesis, I build on existing knowledge of sexual behaviour and its influence on fertility, with a focus on female responses to sexual coercion and on commercially relevant insights in broiler breeders. I use a mix of approaches, from a theoretical model, to an experiment on a captive population of junglefowl, to translational research in broiler breeders. The findings of this thesis add new detail to our understanding of female resistance against sexual coercion, both in this species and in general, and inform suggestions for husbandry optimisations that could be trialled in broiler breeders.

Introduction

"I decided to dedicate myself to sex when I realised that nothing in life is more important, more interesting- or more troublesome. […] If not for sex, much of what is flamboyant and beautiful in nature would not exist."

- Olivia Judson, *Dr Tatiana's Sex Advice to All Creation: The Definitive Guide to the Evolutionary Biology of Sex* (2002)

Sexual selection

This is a thesis about sexual selection. Sexual selection is an extension or special case of natural selection: the change in gene frequencies (or the frequency of any other heritable element) within a population over time, due to differential survival and reproduction (Gregory 2009; Shuker and Kvarnemo 2021; West-Eberhard 2014). When Charles Darwin first outlined the theory of evolution by natural selection in *On the Origin of Species* (Darwin 1859), the process was thought of largely as a struggle against hostile natural forces and a scramble for the resources needed for survival. Sexually selected ornamental traits, then, presented a significant challenge to the theory. A peacock's tail, for instance, brings no conceivable benefit in terms of survival - in fact, it may be a hindrance to survival. Darwin's second great insight, developed a decade later in *The Descent of Man, and Selection in Relation to Sex* (Darwin 1871), provided an answer to this conundrum. To reproduce sexually, individuals combine their gametes with the gametes of conspecifics. Therefore, to be genetically represented in future generations, a member of a sexually reproducing species must not only compete with conspecifics for a limited pool of resources for survival, it must also compete for a limited pool of reproductive opportunities with potential sexual partners. It is this competition for reproductive opportunities that gives rise to *sexual*, as opposed to natural, selection (Shuker and Kvarnemo 2021; West-Eberhard 2014).

Sexual selection can produce adaptations that may seem perverse (costly ornaments that hinder survival, for example) if we consider selection purely as arising from differential survival or from differential reproductive ability in the absence of competition (Lindsay et al. 2019). Sexual selection is, by definition, a form of "soft" rather than "hard" selection (Wallace 1975): the selection pressure is produced by conspecifics (though it can be modulated by other factors), rather than by heterospecifics or the physical environment. It is this competition with conspecifics which produces many of the interesting characteristics of sexual selection. Competition for reproductive opportunities means that an individual might have greater success if it were not for the presence of competitors, and that success is a zero-sum game: one individual attaining more partners, or partners of a higher quality, means the number and quality of potential partners available for others may be reduced. Even if the *number of sexual partners* is not a zero-sum game (because individuals may mate multiply), their reproductive potential (gametes, gestational ability, *et cetera*) remains finite, meaning there are a finite number of tickets to the next generation available. If there were not *competition* for mating opportunities, then there would be no need to signal anything more than one's whereabouts to potential partners, as opposed to elaborate and costly signals of one's superior quality compared to others (West-Eberhard 2014).

One of the most striking consequences of sexual selection is the evolution, in almost all sexually reproducing species, of two sexes, that is, two complementary reproductive roles associated with the production of one of two distinct types of gamete. The ancestral state of sexually reproducing species is thought to be isogamy: the existence of just a single type of gamete. The evolution of anisogamy- where there are (in most cases) two types of gamete, and each can only combine with another of the opposite type- is thought to occur where reducing the size of gametes allows some individuals (i.e. males) to produce more of them, gaining an edge over competitors in fertilisation probability, but forces their sexual partners (i.e. females) to produce fewer, larger gametes in compensation in order to ensure that zygotes are properly provisioned (Billiard et al. 2011; Lehtonen, Kokko, and Parker 2016; Parker, Baker, and Smith 1972; Parker 1982). This is an example of an evolutionary ratchet: "a bizarre, inefficient, exploitative creativity that, once embarked upon, became very nearly inescapable" (Hrdy 1981).

In many species, including almost all vertebrates, each individual is capable of reproducing via one sex role only at a time, or during their lifetime (sequential hermaphroditism or gonochorism respectively, as opposed to simultaneous hermaphroditism, where individuals simultaneously have the ability to reproduce via both sex roles). This facilitates the evolution of specialised anatomical structures and behaviours that support reproduction via one of the two roles (Schärer, Rowe, and Arnqvist 2012). Females have evolved to provide parental investment beyond the initial investment of large gametes: they very often provision young via gestation, lactation, or the production and incubation of resource-rich eggs, and often provide the bulk of parental care. While males may provide parental care, and while there exist some examples of sex-role reversed species where males gestate young (Avise et al. 2002; Jones et al. 2005), these roles mean that, across most sexually-reproducing organisms, the physiological reproductive capacity of females is generally the limiting factor for the rate of production of offspring, and that males can generally be thought of as in competition with each other to attach their genes to the reproductive potential of females (Janicke et al. 2016).

A male's reproductive success can be thought of as the product of his number of sexual partners, his paternity share with each partner, and the mean fecundity of his partners (McDonald et al. 2017). A female's reproductive success, however, is limited by personal fecundity and does not have the potential to be dramatically multiplied in this way by increasing the number of partners. Female fitness is influenced by the quality of her sexual partners, and females compete among themselves for access to, and monopolisation of, high-quality mates (Forsgren 2011; Rosvall 2011); high-quality males being those who, for instance, possess “good genes” which may be inherited by offspring (Hamilton and Zuk 1982; Zahavi 1975), or those who may provide direct benefits to the female such as nuptial gifts (Arnqvist and Nilsson 2000).

Many early researchers assumed that this difference meant females would not benefit from polyandry (copulation with more than one male) and were expected to favour monogamy. This belief dates back to Darwin, who famously noted that “males are almost always the wooers” of “coy” females, though he did not provide a full explanation for this observation (Darwin 1871; Hrdy 1986). An explanation was later to come in the form of Angus Bateman’s experiments in *Drosophila*, which reported that in males, variance in reproductive success was larger, and showed a stronger association with the number of mating partners, compared to females (Bateman 1948) (though the methodology and interpretation of this particular paper has been much dissected: see Sutherland, 1985; Dewsbury, 2005; Snyder and Gowaty, 2007). This result cemented for several decades the view that males gain the most fitness by indiscriminately copulating with as many females as possible, and females by being highly selective, copulating with the highest quality male available and no others (Dawkins 1976; Trivers 1972).

However, more recently it has become clear that not only do females of many species actively solicit copulations from multiple males, but that their offspring may be sired polyandrously (i.e. by multiple males), even in species with social monogamy (Parker and Birkhead 2013; Pizzari and Wedell 2013; Westneat 1987). An important consequence of this is that intra-sexual competition can continue after copulation, as the sperm of different males compete to fertilise a female’s ova (Parker 1979). This post-copulatory competition, known as “sperm competition”, can be just as significant or more significant in some contexts than pre-copulatory competition in determining an individual’s reproductive success (Marie-Orleach et al. 2016; McDonald et al. 2017). Sperm competition creates selection on males to invest in large, high-quality ejaculates (Parker and Pizzari 2010), to tailor their ejaculate investment plastically depending on factors such as the attractiveness and promiscuity of females (Pizzari et al. 2003), and to decrease the level of post-copulatory competition they are subject to, via tactics such as mate guarding (Rowe 1992). In addition, females may be able to bias the outcome of post-copulatory competition towards preferred males, through a phenomenon known as “cryptic female choice”, though this has been less explored and consequently is little understood (Firman et al. 2017).

Many benefits of polyandry to females have been proposed, ranging from insurance against male infertility to obtaining parental investment, nuptial gifts and beneficial ejaculate compounds from multiple fathers (Hrdy 1977; Jennions and Petrie 2007; Nason and Kelly 2020; South and Lewis 2011; Wedell, Gage, and Parker 2002; Worthington and Kelly 2016). However, a comprehensive theory of the fitness costs and benefits of polyandry to females in different settings is lacking. Since polyandry has such significant consequences in shaping the operation of sexual selection, such an understanding would be valuable (Collet et al. 2012; Pizzari and McDonald 2019; Price et al. 2014).

The Darwin-Bateman paradigm of eager males and chaste females may be an oversimplification that fails to predict certain phenomena (Ah-King 2013; Dewsbury 2005), but as a general rule it is not wrong to say that males benefit more than females from the acquisition of multiple mating partners (Janicke et al. 2016). Although females can benefit from polyandry, empirical evidence suggests that the optimal number of sexual partners for females is often "intermediate": greater than monogamy, but lower than the optimal number of partners for males (Arnqvist and Nilsson 2000; Slatyer et al. 2012).

This discrepancy between the optimum copulation rates of males and females creates widespread sexual conflict, where females and males have different optimal outcomes for an interaction, and only one of these outcomes can occur. Copulation has different fitness costs and benefits to males and to females, and there are many situations where copulation is expected to benefit the fitness of males, but harm the fitness of females (Chapman et al. 2003; Clutton-Brock and Parker 1995). Therefore, males are expected to gain a fitness benefit if they can force females to copulate. Females, on the other hand, benefit by preventing forced copulations, or by preventing them from resulting in fertilisation; they benefit both in terms of preventing costly excess copulations and in retaining control of paternity. Across the animal kingdom, we see a variety of physiological and behavioural adaptations to this conflict (Arnqvist and Rowe 2002; Clutton-Brock and Parker 1995). For instance, sexual conflict over mating is thought to be a significant driver of genital coevolution across many taxa (Brennan et al. 2007; Brennan and Prum 2015; Eberhard 1985; Orbach et al. 2017).

Conflicts are inevitable when two unrelated individuals engage in the joint endeavour of sexual reproduction, since the interests of the two are seldom perfectly aligned. Conflicts occur not only over whether to copulate, but over many aspects of reproduction: how much investment each parent makes in offspring, for instance, or whether females remate (Chapman et al. 2003; Trivers 1972). Intersexual conflict can depress population fecundity, often because tactics that benefit individual males do so at a cost to other males and to females (Coulson et al. 2007; Dean et al. 2010; Holland and Rice 1999; Hrdy 1977; Long et al. 2009; Warner et al. 1995). Understanding sexual conflict is therefore of significance for the management of farmed animal populations, both in terms of maximising productivity (the growth rate and reproductive rate of individuals in the population) and minimising welfare concerns arising from conflict (Ellen et al. 2014; Tommaso Pizzari 2016). Sexual conflict is also relevant for the management of endangered populations, since negative population growth may be exacerbated by sexual conflict: an evolutionary tragedy of the commons (Rankin, Dieckmann, and Kokko 2011). In both cases, the interests of those responsible for managing the population are at odds with the interests of some of the individuals within the population, which may include increasing their own fitness to the detriment of other individuals in the population.

Why study chickens? The fowl as model system

The domestic chicken (*Gallus gallus domesticus*) has been a consistently present character throughout the history of research into animal behaviour and sexual selection. Long before the significance of polyandry in creating postcopulatory competition was recognised, the fact that domestic chickens mate multiply was noted by Aristotle: the first known report of this behaviour in birds (cited in Birkhead, 2000; Pizzari and Wedell, 2013). Domestic chickens were the first species in which dominance hierarchies were formally described and studied, and the term “pecking order”, now in common colloquial usage, derives from these early observations (Strauss et al. 2022). And it was from an anecdote about US President Calvin Coolidge and a libidinous chicken that the term “Coolidge effect” (i.e renewed sexual interest with a novel mate) is derived (Dewsbury 1981), an effect which is the subject of one of the data chapters of this thesis.

Today’s chickens were domesticated from junglefowl (*Gallus gallus subspp*), predominantly red junglefowl, around eight thousand years ago (Eriksson et al. 2008; Fumihito et al. 1996; Tixier-Boichard, Bed’Hom, and Rognon 2011). Junglefowl are a medium-sized, omnivorous species in the order Galliformes (game birds), living in densely forested regions of southeast Asia. Red junglefowl live in groups of up to around forty individuals, exhibiting fission-fusion dynamics similar to those of some social mammal species such as primates, and have a polygynandrous mating system, with no pair bonds and females providing all parental care (Collias and Collias 1967; Mcbride, Parert, and Foenander 1969; Pizzari and McDonald 2019).

Domestic chickens retain many of the same behavioural patterns observed in red junglefowl. However, some aspects of their behaviour and physiology have been altered by domestication. Some changes have been directly selected for, while others are likely to be pleiotropic effects as part of a domestication syndrome (Jensen 2014; Karlsson et al. 2015; Schütz et al. 2002). Most noticeably, they are larger: the size of domestic chickens varies by breed, but most weigh between 2-5 kilograms, while red junglefowl weigh approximately 800-1500 grams (with males significantly larger than females). This change means that the flying capability of domestic chickens is reduced compared to junglefowl: neither is capable of sustained flight, but junglefowl can easily fly several metres to evade predators, which most domestic chickens cannot. In addition, while junglefowl lay discrete clutches of eggs during the breeding season, domestic chickens have been selected for higher egg production and generally lay eggs on a near-daily basis throughout the year, something which no wild bird does. Since junglefowl are wild animals with many natural predators, they show an aversion to humans (Collias and Collias 1996), devote large amounts of time to vigilance, and utter alarm calls in response to potentially threatening stimuli (Wilson et al. 2008). These fearful behaviours have been reduced as part of domestication (Elfwing et al. 2014; Karlsson et al. 2015). As is typical of domesticated animals, domestic chickens have smaller brains relative to body size than their wild ancestors, though different regions of the brain show different proportional changes (Katajamaa et al. 2021).

Since their initial domestication, there has been significant introgression between wild junglefowl and domestic chickens, and there may be few if any remaining populations of "pure" wild junglefowl with no genetic contribution from domestic chickens (Nguyen-Phuc and Berres 2018). In addition, there are many established feral populations of domestic chickens across the world, which have reverted to a free-living state (Henriksen, Gering, and Wright 2018). All of these are used as model species for sexual selection research: junglefowl and feral fowl give a more natural view of the species' behaviour, while domestic chickens have the benefit of being easily accessible and of direct commercial relevance to the poultry industry (Pizzari and McDonald 2019).

The fowl as a model system represents an interesting counterpoint to the majority of bird species, which have a mating system based on pair bonding (Birkhead and Møller, 1992; Pizzari and McDonald, 2019). The fowl therefore provides a "purer" view of competitive processes, since every individual can in theory copulate with every other individual without the constraint of social monogamy. This means that reproductive success can show a natural distribution with high levels of variation, as opposed to systems with social monogamy, where the distribution may be flattened to some degree (extra-pair copulations notwithstanding). The networks of sexual interactions in this species are dense, with many individuals having many sexual partners. There is large variation in the number of sexual partners, resulting in high levels of unevenness in the distribution of reproductive success, with some individuals producing many more chicks than others, especially among males (Collias and Collias 1996). The high level of polyandry shown by females of this species means that postcopulatory competition is a significant factor in determining male reproductive success, and males dynamically adjust their ejaculates in response to factors affecting this competition (Cornwallis and Birkhead 2006, 2007a; Pizzari et al. 2003).

The reproductive physiology of fowl is fairly typical of birds (Assersohn, Brekke, and Hemmings 2021; Birkhead 2016). Males lack an intromittent organ; instead, their ejaculate is released from the cloaca, and deposited onto the female cloaca during copulation. During copulation, which lasts for just a couple of seconds, the male "mounts" (stands on the back of) the female, often steadying himself by grasping her comb or the feathers of her head and neck in his beak. Females may assist by raising their tails and presenting their cloacae. However, this remains a somewhat challenging balancing act for the male, since the cloacae of both sexes are not positioned for ease of contact, and may be difficult to correctly locate as both are small openings buried in feathers (Pizzari and McDonald 2019).

After insemination, sperm travel up the oviduct of the female, where they may be stored in minute invaginations of the mucosal lining known as sperm storage tubules at the utero-vaginal junction of the female oviduct, structures which are widespread among birds (Birkhead and Møller, 1992; Birkhead and Møller, 1993; Assersohn, Brekke and Hemmings, 2021). Females possess a single ovary which, during the laying season, releases one mature ovum per day. In birds, ova are very large: they are the yolks of eggs that we eat, with a pronucleus on the surface available for fertilisation (Birkhead 2016). This yolk sustains an embryo throughout its development within the egg until it hatches, and represents a very large nutritional investment in offspring on the part of females (Birkhead 2016). After being released, the ovum then travels down the oviduct, where albumen, membrane and shell are deposited, before it is eventually laid (Birkhead 2016).

If fertilisation is to occur, it must occur during a brief time window immediately following the release of the ovum, before additional layers begin to be added. The storage of sperm is thought to ensure that sperm can be present at the correct moment, and allows copulation and fertilisation to be chronologically decoupled (Birkhead and Møller 1993; Hemmings et al. 2015). Female fowl have the ability to maintain fertile sperm in their storage tubules for around two weeks, though the number of sperm stored, and consequently the probability of fertilisation, decreases with time since insemination (Wishart 1987). Like most birds, this species shows "last male precedence": that is, when a female copulates with multiple males, the most recent male is more likely to sire chicks. This is consistent with results expected due to passive loss of sperm from storage tubules over time (Birkhead, Wishart, and Biggins 1995; Lessells and Birkhead 1990).

Sperm storage intensifies the potential for post-copulatory competition, since a given male's ejaculate may have to compete with the ejaculates of other males with whom the female copulated at any time within an extended window both before and after his own copulation (Birkhead and Møller 1993). Only a small fraction of inseminated sperm are taken up into the storage tubules (Brillard 1993), and it is thought that some kind of active selection by females contributes to determining which sperm are taken up; for instance, only motile sperm are taken up (Bakst, Wishart, and Brillard 1994). Males with higher sperm mobility sire a larger proportion of offspring in sperm competition assays, and this is thought to be in part due to biased uptake into sperm storage tubules (Birkhead et al. 1999; Froman et al. 1999). However, the mechanisms of uptake, maintenance, and release of sperm within the female oviduct remain poorly understood (Hemmings et al. 2015; Holt and Lloyd 2010; Zavaleta and Ogasawara 1987).

One distinctive feature of the fowl as a model system is the importance of dominance hierarchies. Dominance hierarchies in domestic chickens were first described a century ago by Norwegian zoologist Thorlief Schjelderup-Ebbe, who coined the term "Hackliste" (German for "pecking order") (Strauss et al. 2022), the idea being that aggression flows directionally through groups of chickens, with birds higher in the pecking order aggressing those lower down more than *vice versa*. This system reduces the level of aggression among individuals competing for resources, since individuals know in advance when they are outranked and would do better to acquiesce (Estevez, Newberry, and Keeling 2002; Hobson, Mønster, and DeDeo 2021). Dominance hierarchies are not always linear, however: circular relationships have also been described in this species, where A is dominant over B, and B is dominant over C, but C is dominant over A (Pizzari and McDonald 2019).

Dominance hierarchies are sex-specific, with separate hierarchies for females and for males. In males, dominance is a phenotypic trait with a very strong association with reproductive success (Guhl and

Warren 1946; McDonald et al. 2017). Dominant males copulate more often, copulate with a larger number of partners, and have a greater degree of mate monopolisation (Johnsen, Zuk and Fessler, 2001; McDonald *et al.*, 2017); they seem to both be preferred by females and also to be better able to aggressively exclude other males (the latter of which may be to the detriment of females, forcing them to be less polyandrous than would be optimal) (Dean et al. 2010; Warner et al. 1995). There is some evidence that subordinate males may invest more in ejaculates to mitigate the loss of reproductive success that they suffer due to exclusion from copulation opportunities (Froman et al. 2002; Pizzari, Cornwallis, and Froman 2007). Dominant females, on the other hand, outcompete subordinates for preferred feeding, nesting, perching, and dust-bathing sites (Wang et al. 2022), and are able to invest more heavily in offspring, rearing more chicks (Collias, Collias, and Jennrich 1994) and laying heavier eggs (Pizzari et al. 2003). Dominant females are often less polyandrous than subordinate females (McDonald et al. 2019); however, it can be difficult to distinguish effects of female dominance on mating interactions from effects of age, since older females are usually dominant over younger females (Kim and Zuk 2000; McDonald et al. 2019).

Both males and females possess fleshy sexual ornaments - combs and wattles - whose size and redness is an indicator of social rank. Larger, redder combs are weakly associated with high social rank (Zuk and Johnsen, 2000; Johnsen, Zuk and Fessler, 2001; Carleial, McDonald and Pizzari, 2020), and seem to enhance the sexual attractiveness of both males and females (Cornwallis and Birkhead 2007b; Ligon and Zwartjes 1995; Pizzari et al. 2003). However, this effect is highly socially dependent (Johnsen, Zuk and Fessler, 2001; Parker and Ligon, 2002), and can be difficult to disentangle from the effects of social rank *per se*.

Sexual conflict over mating is high in this species. Females are subject to high levels of sexual harassment, and a large proportion of copulations are forced. Despite this, females retain some agency over copulation. Females solicit copulations from preferred males, socially associate with dominant males (leading to more copulations), and have some success in resisting attempts at forced copulations (Løvlie and Cornwallis 2005; Løvlie and Pizzari 2007). The uttering of distress calls by females can prompt other males to intervene and prevent a forced copulation from occurring (though the intervening male will often then copulate with the female) (Løvlie, Zidar, and Berneheim 2014; Pizzari 2001). In addition, females may bias paternity following copulation. Females have been observed ejecting sperm from their cloacae following copulation, and are more likely to eject sperm from subordinate males (Pizzari and Birkhead 2000). The fact that females of this species can store sperm internally for up to two weeks between copulation and fertilisation creates the potential for mechanisms of cryptic female choice, such as biasing the uptake of sperm into storage tubules, its maintenance, or release, although there has been little in the way of direct investigation into these possibilities. In general, though many mechanisms of female control over copulation and fertilisation have been either proposed or demonstrated in this species, a complete view of how these are utilised in different situations is lacking.

Broiler breeders

Over the past fifty years, the number of farmed chickens in the world has increased approximately fivefold (Mottet and Tempio 2017), making chickens an order of magnitude more numerous than any wild bird species (Callaghan, Nakagawa, and Cornwell 2021). The size of the poultry sector is projected to increase further in the coming years, both in absolute numbers of birds and relative to other farmed animals, with much of this increase driven by demographic and economic growth in the developing world (Alexandratos and Bruinsma 2012; Kearney 2010). Chickens are an extremely efficient species to farm, with rapid maturation times, high feed conversion ratios, and tolerance for a wide range of environmental conditions (Alexandratos and Bruinsma 2012; Febrer et al. 2006; Gerber, Opio, and Steinfeld 2007).

Alongside this expansion in the scale of poultry farming, intensive artificial selection has significantly altered the phenotypes of these animals as compared to traditional lines of domestic chickens. Broilers (chickens raised for meat) are now capable of an explosive rate of growth, growing to several times the size of their predecessors, reaching their full size within less time, and requiring less feed to do so (Havenstein, Ferket, and Qureshi 2003; Zuidhof et al. 2014).

The broiler industry has a pyramidal structure, with increasing numbers of individuals in each generation (Pollock 1999). At the top of the pyramid are a relatively small number of pedigree birds, maintained by breeders in a highly deliberate regime of selection and interbreeding. Their descendents are broiler breeders, whose commercial purpose is to produce hatching eggs in order to multiply the numbers in each generation. Broiler breeders are kept in large mixed-sex flocks where they are able to mate freely. As opposed to broilers, which are fed *ad libitum* to maximise growth during a short time frame, broiler breeders are fed a restricted diet calculated to maintain health for their longer intended lifespan (Millman and Duncan 2000). Several generations down the pyramid are broilers, which reach full size within weeks and are slaughtered and eaten.

This system means that a single pedigree hen might have over a million descendants (Pollock 1999). Therefore, the fertility of broiler breeders is of enormous significance to this industry, since incremental improvements in fertility might result in substantial gains in efficiency.

However, although genetic selection has resulted in substantial directional changes in physical traits, such as body size, growth rate, and egg output, the hatchability of eggs has plateaued (Laughlin 2008). In addition, many lines of broiler breeders are plagued with characteristic problems of low fertility, and fertility in all lines declines as birds age: these are problems that genetic selection is of limited use in addressing. This may be because fertility is only moderately heritable and is in large part a function of the social environment (Barbato 1999; Laughlin 2008; Pizzari 2016). This makes intuitive sense, since artificial selection pressures work by creating or strengthening an association between a desired trait and reproductive success, whereas high reproductive success is by definition already naturally selected for. Approaches to improving flock productivity should therefore focus on understanding and mitigating whichever physiological or social factors might impede fertility, for instance by managing flocks so as to minimise depressed fertility due to sexual conflict (Pizzari 2016; Rankin, Dieckmann, and Kokko 2011).

The determination of individual reproductive success in this species is complex. Few traits have been shown to have a strong, consistent association with reproductive success: though many phenotypic traits have been investigated, the evidence is mixed and context-dependent (Johnsen, Zuk and Fessler, 2001; McGary, Estevez and Bakst, 2003; Bilcik, Estevez and Russek-Cohen, 2005; Duncan, 2008). One trait which has been consistently linked to male reproductive success is dominance (Guhl and Warren 1946; McDonald et al. 2017). Dominance itself seems to be somewhat heritable (Craig, Ortman, and Guhl 1965). However, it is not possible to increase average dominance within a population, since dominance is a relative social trait and any individual increases are zero-sum; that is, they come at the expense of another individual who is now lower ranking in comparison.

Fertility of broiler breeders may be impacted by the artificial selection regimes to which they have been subject. In general, we expect selection for rapid growth to be associated with trade-offs (Schütz et al. 2002). High body weight seems to show a genetic correlation with some musculoskeletal disorders in broiler breeders (Kapell et al. 2012), which may impair fertility due to reduced locomotor ability (Dawkins and Layton 2012; Duncan, Hocking, and Seawright 1990). Selection for body weight may also exert conformational changes on the rest of the body, which may impede copulation. For instance, a negative relationship between fertility and pelvis width has been reported in broiler breeders (McGary, Estevez, and Bakst 2003; Wilson et al. 1979). However, there is little relationship between weight and fertility on an individual level; in fact, some authors report a positive relationship between the weight of male broiler breeders and their copulation rate (Duncan, Hocking, and Seawright 1990; Hocking and Bernard 1997), which could be due to a relationship between body weight and dominance. Because the various factors that potentially influence individual reproductive success are so intertwined, we have little understanding of how they may interact or how they act via different components of reproductive success.

The commercial environment may also impact the sexual behaviour and feritlity of broiler breeders. Broiler breeders are kept in flocks much larger than their natural group size, and too large for stable dominance hierarchies to form (Pagel and Dawkins 1997). Their environment is of low complexity (Estevez 2009), they feed once per day at a fixed time instead of continuously, and there is no variation in age within the flock. All of these factors represent departures from the environment in which these birds naturally live, and all might considerably impact sexual behaviour and fertility. However, most research on broiler breeders takes place within experimental flocks that are at least one order of magnitude smaller than commercial flocks. Little is known about the sexual dynamics of broiler breeders in a commercial environment, despite the huge economic significance this understanding would have.

Aims of this thesis

This thesis builds on existing knowledge of sexual selection in junglefowl and broiler breeders, with a focus on female strategies in response to sexual conflict over mating, and on commercial applications for better understanding fertility in broiler breeder flocks. I use a variety of approaches, ranging from a theoretical model, to experimental research on a naturalistic population of junglefowl, to highly translational research on broiler breeders.

How do females influence copulation rates under intense sexual harassment? What are the costs and benefits attached to copulation, and to the resistance of copulation attempts? I explore these questions, first through a theoretical model that predicts optimal female responses to sexual harassment under a range of ecological conditions (**Chapter One**), and then through an experiment on junglefowl that investigates female responses to male sexual novelty (**Chapter Two**).

Next, I move onto the factors affecting copulation success and reproductive success in broiler breeders.

Chapter Three is a bridge between basic and applied science, looking at the effect of intrasexual competition on the opportunity for selection in males. It is known that male broiler breeders can vary substantially in reproductive success. But how much of this is due to competition with other males, as opposed to intrinsic variability between individuals? And how much can be explained by the effects of chance, or by the influence of females they copulate with? These questions are investigated using a dataset containing parentage for thousands of pedigree broiler breeder chicks, as well as information on the behaviour of individual males.

Finally, **Chapter Four** explores sexual behaviour on a group level, looking at the interplay of male and female behaviours, their influence on fertility, and their variation from group to group, via behavioural observation of commercial flocks of broiler breeders and assays of their fertility, with the aim of informing optimal husbandry practices.

The experimental work in chapters two, three, and four was approved by the Animal Welfare Ethical Review Board (AWERB) of the Department of Zoology. Chapter one does not involve animal experimentation so no approval was needed.

Chapter 1: Female strategies in response to sexual coercion

"When the final result is expected to be a compromise, it is often prudent to start from an extreme position."

- John Maynard Keynes, *The Economic Consequences of the Peace* (1919)

Abstract

Sexual conflict over copulation is widespread and occurs where copulation benefits one sex, but not the other. Optimal copulation rates of males are typically higher than those of females, stimulating the evolution of behavioural and anatomical adaptations in females to resist male sexual coercion and prevent excess copulations beyond a female optimum. However, resistance to male attempts is costly, and females likely face limits on the efficacy of resistance. Given the incomplete effectiveness of resistance, it may pay a female to begin to resist before she has received her optimum number of copulations in order to pre-empt an expected number of forced copulations that she is unable to avoid, a behaviour we call "compensatory resistance". Here, we present a mathematical model that predicts when females should optimally begin resisting, taking into account the costs of resistance and its expected effectiveness. This model quantifies the level of resistance expected, and predicts four mutually exclusive classes of strategic response from females, depending on ecological parameters. Our results predict that females will engage in compensatory resistance across a range of biological scenarios, with implications for the interpretation of sexual behaviour and the co-evolutionary diversity of sexually antagonistic traits.

Contributions

The chapter was conceived by Ellen Pasternack. Mathematical development of the idea was by Ellen Pasternack and George Johnson. The chapter was written by Ellen Pasternack with input from Eleanor Bath, Grant McDonald, and Tommaso Pizzari.

Introduction

Sexual conflict over copulation

Intersexual conflict over copulation arises because of the difference in reproductive optima of the sexes (Chapman et al. 2003; Parker 2006). A central arena for this conflict is the frequency at which individuals copulate.

Copulation has fundamental benefits to individuals, chiefly, the chance to produce offspring; but can also incur substantial cumulative costs, particularly among females who more frequently suffer somatic damage (Lange et al. 2013). A large body of evidence suggests that copulation per se can represent a significant fitness cost (Rice 2000). Copulation has a negative effect on lifespan in multiple model species such as mice (Garratt et al. 2020), the seed beetle *C. maculatus* (Jigisha et al. 2020), *D. melanogaster* (Fowler and Partridge 1989), and *C. elegans* (Gems and Riddle 1996). Females in particular may suffer somatic damage as a result of copulation. Males in many species possess genitalic spines and devices which wound females (reviewed by Lange et al. 2013) and have been shown to reduce female lifespan (Crudgington and Siva-Jothy 2000). Further, exposure to seminal fluid can be deleterious to female fitness as it can disturb the micro-ecological balance of the reproductive tract, transfer infections, summon a costly immune response, and damage future reproductive fitness (Gordon, Wolfner, and Lazzaro 2022; Rowe et al. 2020; Sheldon 1993; Westneat and Rambo 2000). Seminal fluid also contains male-generated compounds that have been shown to reduce longevity in *Drosophila* (Chapman 1992; Wigby and Chapman 2005) and more widely (South and Lewis 2011). Finally, copulation requires time that could otherwise be spent on other activities (Rowe 1992), and in some contexts may increase individuals' risk of predation via decreased vigilance and increased conspicuousness (Sih, Krupa, and Travers 1990).

The optimum copulation rate for an individual is located where the balance between these costs and benefits derives the highest net benefit in terms of fitness. Since costs and benefits of copulation are likely to systematically differ between females and males in any given species, we expect the optimum number of copulations also to systematically differ for females and males. This produces sexual conflict since only one of these optima can be realised in decisions concerning two individuals. These conflicts have wide-ranging consequences for population dynamics, speciation, and evolution (Chapman et al. 2003; Gavrilets 2000; Parker 2006).

Theory predicts that the optimum copulation frequency is likely to be higher for males than for females in many species (Bateman 1948; Jones 2009; Trivers 1972). For males, number of offspring may be constrained in many cases by access to sexual partners, whereas for females, the number of offspring is more likely to be constrained by physiological limits on fecundity. Females can derive fitness benefits from polyandry in slightly more subtle ways, often by increasing quality, rather than quantity, of offspring (Jennions and Petrie 2007; Parker and Birkhead 2013). Empirical evidence suggests that, as a general principle, the optimum copulation frequency for females is often higher than monogamy, but lower than the optimum frequency for males (Arnqvist and Nilsson 2000; Jones, Arguello, and Arnold 2002; Slatyer et al. 2012). On this basis, we can predict that there will be many cases in which copulation benefits males, but not females, leading to sexual conflict.

Adaptations to sexual conflict over copulation

Due to the difference in optimal reproductive strategies between the sexes, it is possible for sexually antagonistic selection to favour the evolution of sex-specific traits that benefit one sex at the expense of the other (Chapman et al. 2003; Coulson et al. 2007; Parker 2006). As a result of sexual conflict over copulation, selection favours males who are able to sexually coerce females, since males who coerce females are more likely to reach their optimum number of copulations and thus have a selective advantage over non-coercing males. Coercion may occur through direct force, intimidation, or through territorial domination of a necessary resource (Clutton-Brock and Parker 1995; Palmer and Thornhill 2000; Ridley 1990). All these forms of sexual coercion are common in a wide variety of animal species (Coker et al. 2002; Mitani 1985; Rowe et al. 1994; Scott et al. 2005; Wrangham and Peterson 1996).

In addition to coercive behaviour, males of some species also show morphological adaptations to conflict over copulation. For many insect species, genital spines and abdominal clasping organs can facilitate copulations with non-cooperative females (Arnqvist and Rowe 1995; Eberhard 1985; Sakaluk et al. 1995; Thornhill and Sauer 1991). In the waterfowl family Anatidae, the size and structural complexity of the male intromittent organ is positively linked to the frequency of forced extra-pair copulations in a given species, suggesting genital elaboration can be an adaptation to overcome female resistance (Coker et al. 2002). Friesen et al. (2013) found that when spines on the hemipenes of the male red-sided garter snake *Thamnophis sirtalis* were ablated, copulation duration was reduced. Conversely, copulation duration increased when the female cloacal region was anaesthetised. Together these results suggest a situation of sexual conflict in which male genital spines aid in prolonging copulation in the face of female attempts to terminate it.

In turn, this widespread sexual coercion places a selection pressure on females to resist copulation (see ***Figure 1***). This resistance may take the form of physically “fighting off” coercive males, or taking up a stereotyped posture or action that makes intromission impossible. This latter form of resistance is especially common in arthropod species, where often copulation is only possible with female cooperation, for instance through opening their abdomens or extruding ovipositors (Eberhard 1985;

Han and Jablonski 2009; Thornhill and Alcock 1983). In waterfowl, the structural complexity of the female genital tract, with corkscrew turns and dead end sacs, appears to be an adaptation against forced copulations, increasing the difficulty for males of achieving forced intromission as well as reducing the probability of fertilisation from such encounters (Brennan et al. 2007; Brennan, Clark, and Prum 2009). Fertilisation may also be prevented by sperm ejection (Pizzari and Birkhead 2000; Pizzari 2004) or by other physiological or biochemical forms of post- copulation female control (Firman et al. 2017).

Arnqvist and Rowe (1995) provide evidence that spines on the abdomens of female water striders have a role in preventing forced copulations, among several other anti-grasping adaptations that have been described in female water strider species (Gagnon, Duchesne, and Turgeon 2012). Alternatively, female resistance may be expressed as patterns of space use to avoid males or social strategies including coalitions with other females (Darden and Croft 2008; Krupa, Leopold, and Sih 1990; McDonald et al. 2019; Pilastro, Benetton, and Bisazza 2003; Shine et al. 2005), or associations with dominant males for protection from lower-ranking males, and utterance of distress calls that prompt intervention by dominant males when females are approached by a lower-ranking male (Clutton-Brock and Parker 1995; Pizzari 2001).

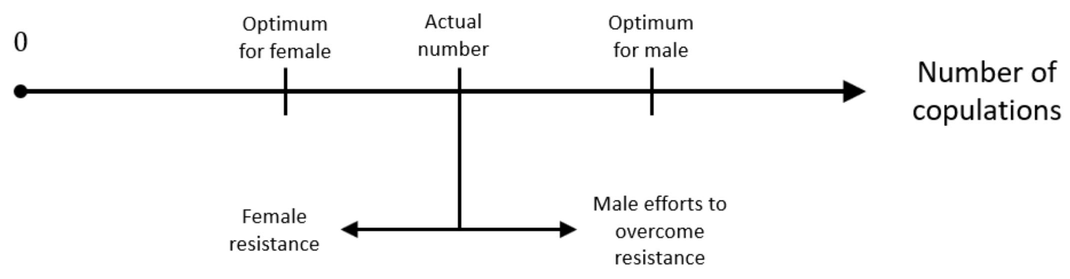


Figure 1: *Graphical illustration of sexual conflict over copulation. Number of copulations is represented on an axis that increases from zero on the left-hand side. An average member of each sex has a different optimum number of copulations. Crudely, the mean number that actually occurs (which is equivalent for both sexes) is expected to lie within the range bounded by these two optima (because if it was outside this range, both sexes would "agree" to increase or decrease it until it returned to the range of conflict). Each sex is expected to use behavioural and physical adaptations to move their actual number of copulations closer to their own optimum, working against the effects of the other sex's sexually antagonistic adaptations.*

Although many theoretical models exist of female resistance to male harms in general (Parker 2006), there is surprisingly little specific to female resistance to attempts at forced copulation. An exception to this is that some authors have produced theoretical models exploring the coevolutionary dynamics and effects on selection of female resistance and male persistence (Gavrilets 2000; Gavrilets, Arnqvist, and Friberg 2001; Kazancioğlu and Alonzo 2012). However, the model detailed in this chapter is the first, as far as we are aware, to attempt to provide a universal model of when females ought to resist copulation attempts.

Benefits of resistance to females

In many contexts, we expect that females could gain a substantial fitness benefit by using resistance to reduce the number of surplus copulations that occur.

Females who are better able to resist sexual coercion are able to keep the number of copulations closer to their own optimum, avoiding excess costs of copulation. This reduction in copulation costs could be considered the "primary" fitness benefit of resistance. In many species, females increase resistance when recently mated, compared to females that either have never mated or are sperm-depleted. This response has been observed in many species of invertebrates, e.g. *Drosophila* (Bastock and Manning 1955; Connolly and Cook 1973); water striders (*Gerridae* sp.) (Lauer 1996; Ortigosa and Rowe 2003); the Heliconius butterfly *Dryas julia* (Boggs 1981); the fly *Ceratitis capitata* and the parasitoid wasp *Muscidifurax zaraptor* (Thornhill and Alcock 1983 p. 131)), as well as in some vertebrates such as feral fowl, *Gallus gallus domesticus* (Løvlie, Cornwallis, and Pizzari 2005). Although manipulation by males is likely to play a role in female resistance to remating (Andersson, Borg-Karlson, and Wiklund 2004; Chen et al. 1988; Eberhard 1985), it also seems likely that females adaptively employ resistance to limit the copulations to which they are exposed, keeping the number closer to their own optimum (Arnqvist 1992; McLean and Stuart-Fox 2010).

In addition, females can use selective resistance, favouring some males over others, as a form of mate choice. This can result in higher quality genetic contribution to her offspring, and also benefits to herself: these benefits arising from selective resistance could be considered the "secondary" benefits. Sparkes, Keogh, and Orsburn (2002) provide empirical evidence suggesting that in the stream-dwelling isopod, *Lirceus fontinalis*, females use selective resistance to favour males that have higher energy reserves. Males with high energy reserves are more likely to be unmated and thus less likely to be sperm-depleted, therefore ensuring females receive adequate sperm numbers. In the marine isopod, *Idotea baltica*, females use resistance to copulation to select males with larger body size (Jormalainen and Merilaita 1995). In red junglefowl *Gallus gallus*, and the domestic chicken *G. g. domesticus*, females show reduced resistance to socially dominant males, with the result that such males have greater reproductive success than subordinate males, fathering a disproportionate number of chicks (Pizzari and McDonald 2019; Wood-Gush 1955).

Female resistance may also have the effect of selecting for males who are able to overcome resistance, causing a process akin to the Fisherian "sexy sons", in which females who resist produce male offspring who inherit increased copulation success from their fathers (Chapman et al. 2003; Eberhard 1985; Gavrilets, Arnqvist, and Friberg 2001; Rowe et al. 1994). However, it is unlikely that benefits associated with mate choice alone are insufficient to select for female resistance; rather, it is thought that female resistance evolves primarily to reduce the costs of copulation, and benefits through mate choice may then arise as a side effect of female resistance (Arnqvist 1992; Chapman et al. 2003; Gavrilets, Arnqvist, and Friberg 2001).

Costs of resistance

Against these potential benefits of resistance, female strategies must weigh up the fitness costs of resistance, which can be large. Resisting copulation attempts can prolong or intensify sexual harassment, resulting in somatic damage or even death (Le Boeuf and Mesnick 1991; Clutton-Brock and Parker 1995; Rowe et al. 1994). Alternatively, resistance may be costly if males dominate access to some necessary resource; females could avoid copulation by avoiding males but at the cost of food or safety (Darden and Croft 2008).

The costs of resistance are likely to have a widespread and significant impact on female sexual strategies. McLean and Stuart-Fox (2010) find that female dragon lizards *Ctenophorus maculosus* decrease resistance when resisting would put them at increased predation risk. “Convenience polyandry” refers to situations where females accept copulations with multiple males, not because of any positive benefit to them of doing so, but because acquiescence is less costly than resistance (Boulton, Zuk, and Shuker 2018; Thornhill and Alcock 1983). Some degree of convenience polyandry has been demonstrated in several species including the water strider model system (Rowe 1992) and in a species of promiscuous marine turtle (Lee and Hays 2004; Wright et al. 2013).

The costs of resistance may be a parameter that is changeable across both short and long timescales. Rowe (1992) argues that the net costs of resistance ought to scale up with the intensity of sexual harassment, because in situations of intense harassment, the successful repulsion of one male may simply mean that a new male takes his place. Additionally, the imposition of high resistance costs may be a strategy that increases male fitness by making it less worthwhile for females to resist, favouring convenience polyandry (Clutton-Brock and Parker 1995).

Effectiveness of resistance

Empirical observations suggest that, across species and across mating systems, the effectiveness of female resistance varies widely. In some species, it is very difficult for fertilisation to succeed without female cooperation, such as in many insect mating systems, where females travel to acoustically signalling males (Ridley 1990; Thornhill and Alcock 1983). In other species, the effectiveness of resistance is much lower, for instance, in wild orang-utans, it was estimated that fewer than 10% of copulation attempts that females resisted were thwarted (Mitani 1985).

Optimal female strategies of "compensatory resistance"

Since resistance is not always totally effective, it ought to be considered as a brake on the increase in copulation number, slowing down copulation rate rather than bringing it to a complete stop. Therefore, we might expect the onset of resistance to occur before the female has attained her optimum number of copulations, in order to avoid overshooting her target number (see ***Figure 2***). Resistance that occurs prior to the female's optimum copulation number being reached is here termed "compensatory resistance", since it compensates for the expected degree to which resistance is ineffective.

However, since resistance is not cost-free, we do not necessarily expect females to begin resistance at the point that would lead to their optimum number of copulations occurring. Instead, we expect the cost of resistance to trade off against the cost of excess copulations. This would mean the optimum point to begin resistance would be one that results in overshooting the optimum number of copulations by a certain amount.

The influences on female resistance are not well characterised; indeed, the fact that the effectiveness of resistance varies, and the potential importance of this, is seldom discussed. Here, we present a model that explicitly considers the effectiveness and the cost of female resistance in order to calculate the optimal amount of resistance under different ecological conditions. We describe how resistance behaviour is expected to vary in response to changes to the model parameters (cost and effectiveness of resistance, and number of attempts females expect to receive), defining four exhaustive and mutually exclusive sets of female strategies that are expected with different ranges of input parameters. Finally, we discuss the implications of this model for the interpretation of observed female behaviour.

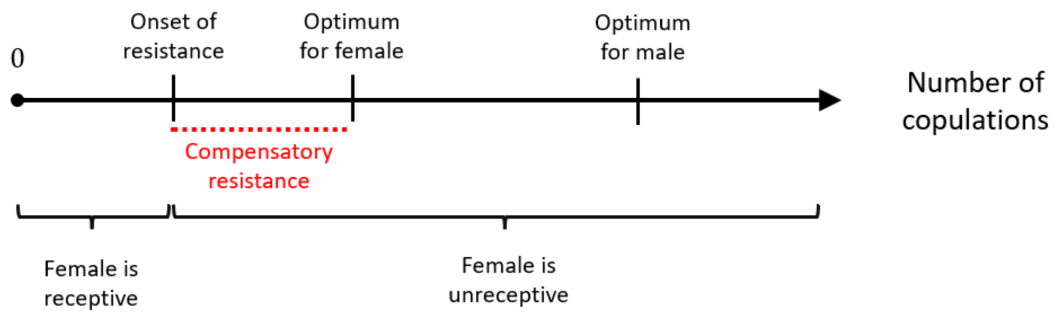


Figure 2: *Diagram showing a copulation strategy in which at a certain point, a female switches from non-resistance to resistance (see caption for* ***Figure 1****). We expect that this switch point should sometimes occur before the female's optimum number of copulations has been reached, because strategies should account for the "leakiness" of resistance; i.e., the fact that some copulations will occur despite resistance. Resistance that occurs before the female's optimum is reached is termed "compensatory resistance".*

Throughout this paper, we refer to females as the resistant sex, since in the majority of cases where there is sexual conflict over copulation it is expected to be the female who has the lower optimum number of copulations. There are however some cases where females experience sperm limitation and thus may actively harass males for copulations (Wedell, Gage, and Parker 2002). We therefore use females as a generalising shorthand for whichever sex has the lower optimum number of copulations.

When should females begin resistance?

In our model, we assume that females begin in a state of non-resistance, before switching to resistance once a threshold number of copulations has occurred (see ***Figure 2***). This is similar to model species such as *Drosophila*, in which females switch from a receptive, non-mated state to a resistant, mated state (Hopkins and Perry 2022). Though in reality, individual females would have to undergo this shift following a non-negative integer number of copulations, our model is not limited in this way: it is a continuous model, so can give non-integer optima; in addition, optima can be negative, which would translate to beginning resistance immediately, after 0 copulations. This allows the model to make fine-grained predictions so that we can understand continuously what is happening, rather than sudden jumps from one integer to the next which may leave the underlying shifts more opaque. The optimum number of copulations is a theoretical value, so it does not need to be constrained to the natural numbers: it makes no less sense to say that an individual's optimum number of copulations is 3.5 as either 3.0 or 4.0.

Let us say there is a fitness payoff of copulation, $P(N_m)$, that is a function of the number of times an individual copulates, N_m. We assume that copulations take place within a large, well-mixed population and thus that each copulation is with a new partner. The function that describes $P(N_m)$ is given by equation **1**, where *A*, *B*, and *C* are positive constants.

$$\textbf{1.} \qquad P(N_m) = A(1 - e^{-BN_m}) - CN_m$$

The exponential term in equation **1** gives a benefit of copulation that saturates towards a maximum *A* at a rate indicated by *B*. The parameter *A* can be thought of as an individual female's maximum possible fecundity in the absence of any copulation costs, and *B* as a saturation rate indicating how quickly a female's maximum fecundity is reached with successive copulations. A low value of *B* would describe a situation where females require multiple copulation partners to realise their fecundity potential *A*, for instance due to sperm limitation or fecundity stimulation. Conversely, a high value of *B* would describe a situation where female fecundity is quickly saturated and does not increase with further copulations. The constant term subtracts a cost *C* with each copulation. For simplicity, the cost is assumed to be the same for each copulation. A non-zero cost of copulation means that the maximum possible fecundity *A* cannot be reached. It also means that instead of reaching a plateau at the maximum possible fecundity, the payoff $P(N_m)$ begins to decline with additional copulations after a certain point. The shape of the payoff function given in equation **1** is plotted in ***Figure 3***.

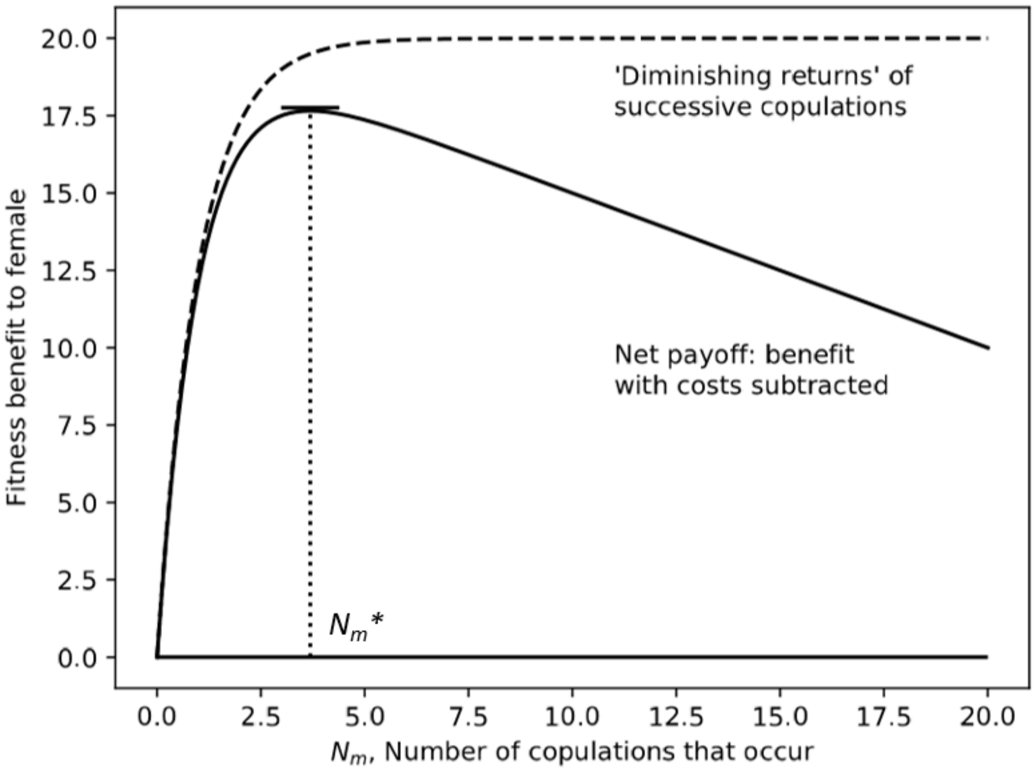


Figure 3: *The solid line shows the shape of the payoff function given in equation **1** where A = 20, B = 1, C = 0.5. Fitness increases with increasing number of copulations, N_m, before beginning to decline as the exponential term saturates and the cost term begins to dominate. The optimum number of copulations, ${N_m}^*$, is defined as the value of N_m that gives the highest payoff according to this function. The dashed line describes the case where C = 0, showing the saturation of the payoff of increasing N_m when there is no cost of copulation.*

By differentiating **1**, the number of copulations that gives the highest fitness payoff, which we will call ${N_m}^*$, will be given by equation **2** (see appendix to this chapter).

$$\mathbf{2} \qquad {N_m}^* = \frac{-1}{B} \ln \left(\frac{C}{AB}\right)$$

${N_m}^*$ is the number of copulations where the costs and benefits balance to give the highest net payoff to the female. If a female had a totally cost-free and effective way to control the number of copulations, then her optimal strategy would be to copulate ${N_m}^*$ times, and a female could be said to have “won” sexual conflict over copulation if this was the number of copulations that actually occurred. However, females usually do not have perfect control of the number of copulations that occur — instead, they can only select the number of advances they accept or resist, and in doing so indirectly influence the number of copulations in which they engage.

In this model, a female expects a certain number of copulation attempts N, which must be partitioned into a number to accept, N_a, and a number to resist, N_r. Following this, the number of copulations that occur, N_m, can be said to consist of the number that are accepted plus the number that are unsuccessfully resisted. If resistance has a probability p of preventing copulation, and has a fitness cost r to the female, then we can apply the payoff function $P(N_m)$ given in **1** to the number of copulations that actually occur, and subtract the cost of resistance, giving the expected payoff of a given partitioning strategy as

$$P(N_a + (1 - p)N_r) - r\,N_r \tag{3}$$

Here, females experience a twofold cost. Firstly, they experience the costs, as well as the benefits, associated with the number of copulations that actually occur, whether they were accepted or resisted. These costs and benefits are calculated by the payoff function $P(N_m)$, which makes up the first part of formula **3**.

Secondly, there is a cost r associated with resisting copulation attempts, whether or not the resistance successfully prevents copulation. The resistance costs are therefore given by rN_r: the cost of resistance, multiplied by the number of times females resist, whatever the outcome.

The higher the value of N_a, the lower the consequent value of N_r (since $N = N_a + N_r$), and the lower the resistance cost rN_r associated with executing the strategy.

Under this model, if r is greater than *C*, then we do not expect to see female resistance under any circumstances, as *C*, the cost of one copulation, is also the maximum possible benefit of resisting one copulation attempt. We can go further and say that we do not expect to see resistance if r is greater than *Cp*, or the cost of one copulation multiplied by the probability that resistance prevents that cost from occurring. In other words, we do not expect resistance to occur if its cost is greater than the expected cost saved.

If we write $(1 - p)$ as q, and N_r as $N - N_a$, then **3** can be rewritten as

$$P(N_a + q(N - N_a)) - r\,(N - N_a) \tag{4}$$

By differentiating **4** with respect to N_a (see Appendix to this chapter), we find that ${N_a}^*$, the value of N_a which gives the greatest net fitness payoff, will be given by

$${N_a}^* = \frac{1}{p}\,\left(\frac{-\ln(k)}{B} - qN\right) \tag{5}$$

Where k is a constant:

$$k = \frac{C - {}^{r}/_{p}}{AB} \tag{6}$$

k is not a quantity that readily lends itself to biological interpretation. Roughly speaking, it is a ratio of the copulation costs expected to be saved by resisting compared to the benefits of copulation. It is not intended to be interpreted directly, but rather is included both as a simplifying shorthand for equation **5** and because the values it takes predict four different cases of the model, which are outlined below.

Cases

This general model can be subdivided into four exhaustive cases given by ranges of values of k, each of which produces distinct model behaviour. These are summarised in ***Figure 4*** and ***Table 1***.

Case 1: Resistance too costly, never resist

Case 1 occurs when there is no solution to equation **5** and therefore no value of ${N_a}^*$. Since it is impossible to take the logarithm of a non-positive number, case 1 occurs for values of k that are below or equal to zero. Given *A*, *B* and *C* must always be positive in order for the model to be applicable (there must be both some cost and some benefit associated with copulation), then rearranging equation **6**, we find that $k \leq 0$ implies $r \geq Cp$. As previously discussed, we do not expect any resistance when this is the case, as it implies that the cost of resistance is greater than or equal to the expected gross cost saved by resisting (cost of one copulation multiplied by the probability that resistance prevents copulation from occurring).

Case 1 describes a situation akin to convenience polyandry. In convenience polyandry, females mate with multiple males because the cost of resisting them is too high to be worthwhile (Rowe 1992). In case 1, females do not attempt to control the number of copulations (which may be with any number of males, though the assumption is that each is with a new male) via resistance because the cost of resistance is greater than the expected cost saved. This can result from any combination of: resistance having a low effectiveness, the cost of copulation being low (so there is little to be gained through resistance), or a high cost of resistance, which may be strategically imposed by males as a form of sexual coercion. In this situation, females “give up” on sexual competition over copulation rate because their best strategy is to cede rather than engaging in a costly tug of war. In case 1, the model does not give an optimum value of N_a because there is no point at which females can optimise their fitness by switching from non-resistance to resistance. We might expect case 1 to occur where females face a high intensity of sexual harassment, where there is a strong disparity in size or strength between the sexes, or where males have territorial control of a resource such as food or nesting sites, all of which might make resistance especially costly and/or ineffective.

Case 2: Insufficient copulation attempts, never resist

Case 2 occurs where ${N_a}^*$, the optimum number of copulation attempts to accept, is greater than *N*, the expected number of attempts a female will receive. Because a female cannot accept more attempts than she receives, it is not possible to execute her optimal strategy of accepting $N_a{}^*$ copulations. Instead, we expect selection to favour a female strategy of "always accept". Rearranging equation **5**, we find that case 2 is produced when $0 < k < e^{-NB}$.

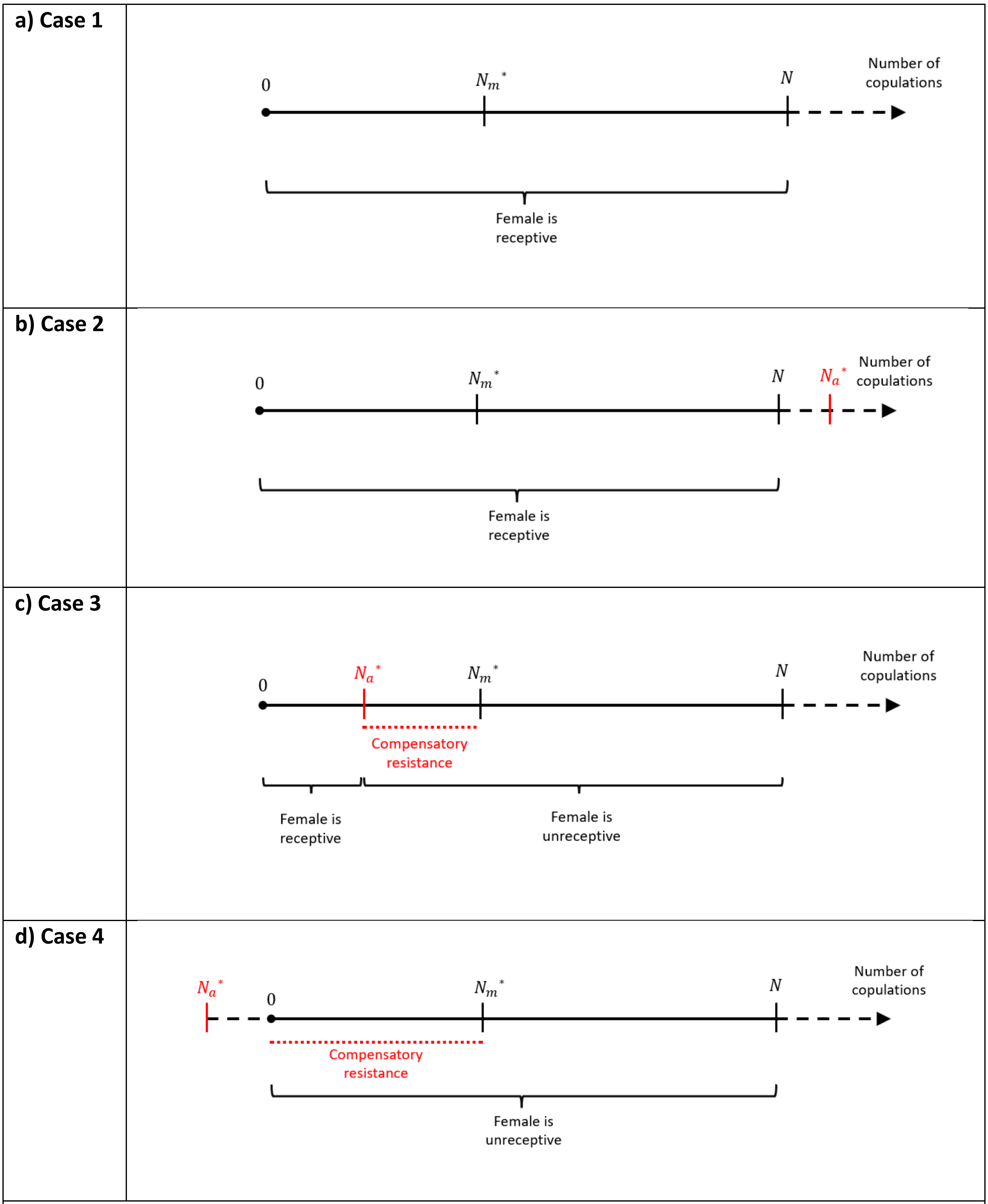


Figure 4: *Illustration of the four possible cases described by this model. Number of copulations is represented on a number line increasing from 0 on the left hand side.* $N_m{}^*$ *represents the optimum number of copulations from a female's point of view. N represents the number of attempts she*

receives. $N_a{}^*$ represents the point where she should switch from non-resistance to resistance for the optimal fitness payoff. Actually realisable values for $N_a{}^*$ are those between zero and N, represented by the solid black number line. Dashed lines below zero and above N represent ranges of $N_a{}^*$ which could not actually be realised as strategies. "Compensatory resistance" refers to any resistance which takes place before a female's optimum number of copulations has occurred. ***4a)*** *shows case 1, where there is no value of $N_a{}^*$ (convenience polyandry);* ***4b)*** *shows case 2, where $N_a{}^* \geq N$, therefore favouring a strategy of 'always accept',* ***4c)*** *shows case 3, where $0 < N_a{}^* < N$, favouring a strategy of switching to resistance once $N_a{}^*$ copulations have occurred; and* ***4d)*** *shows case 4, where $N_a{}^* \leq 0$, favouring a strategy of 'always resist'. See main text for discussion of all four cases.*

This case, where female fitness is constrained by the number of copulation opportunities, might be expected in rare, solitary living species such as large carnivores. Something similar to case 2 may also represent male strategies in many cases. In theory males will also reach a point where the costs of further copulations outweigh the benefits, for instance if male sperm reserves are depleted such that additional paternity from more copulations becomes saturated, while energetic costs of copulation remain. However, in many cases this point may rarely be reached if male sperm replenishment rates greatly exceed the number of copulation opportunities. In such cases selection might favour males who accept all copulation opportunities.

Note that although the behaviour we expect to observe in the female is identical for case 1 and case 2, the underlying pressures on the female are extremely different. In contrast to case 1, where there is no solution, for case 2 a solution to equation **5** does exist; there is a point where, in theory, a female would optimise her expected fitness payoff by switching from non-resistance to resistance. However, this does not actually occur because of a limit in the availability of interested males. In some circumstances it may be possible to experimentally distinguish whether a female showing 'always accept' behaviour is employing a case 1 or case 2 strategy by providing her with additional mates and observing whether a switch to resistance occurs.

In case 1, females 'lose' sexual conflict over copulation, experiencing costly excess copulations, because the costs of attempting to control copulation rate are greater than the benefits. By contrast, in case 2 there need not be any conflict: males and females may both derive a fitness benefit from further copulations but be limited by the availability of sexual partners. The limit to female fitness would in this case be insufficient, rather than excess, copulations. In case 2 a female's receptivity helps her to achieve her optimum number, rather than occurring with no regard to her optimum copulation number, as in case 1.

Case 4: Resistance too ineffective, always resist (Case 3 will be discussed afterwards)

Case 4 occurs where ${N_a}^*$, the optimum number of copulation attempts to accept, is below zero. Since it is not possible to accept a negative number of attempts, the optimum strategy is not realisable and we expect selection to favour a strategy of “always resist”. In this way case 4 could be described as the opposite of case 2. By rearranging equation **5**, we find that case 4 is produced when $k > e^{-qNB}$.

This resistance, occurring before ${N_m}^*$ is reached, is labelled “compensatory resistance”. The female resists not because she has reached the point where further copulations bring more costs than benefits, but because forced copulations, as a result of imperfect or ‘leaky’ resistance, are likely to reach or exceed the remainder of the copulations she requires.

Case 3: Accept ${N_a}^*$ attempts before switching to resistance

Case 3 occurs in the “Goldilocks zone” between cases 2 and 4— so for values of k where $e^{-NB} \leq k \leq e^{-qNB}$. Case 3 is the only case where equation **5** gives a value of ${N_a}^*$ that is actually realisable: a solution to the equation exists, and it is not less than 0 or greater than N. By beginning resistance at this point specified by equation **5**, the female reaches her maximum possible net fitness payoff. This optimum switch point, ${N_a}^*$, is often lower than ${N_m}^*$, the optimum number of copulations, to compensate for some expected level of coerced copulations. However, unlike in case 4, the expected number of coerced copulations is not so high that ${N_m}^*$ would be reached without the female accepting some copulations in addition to those which are coerced. The more effective female resistance is (i.e. the higher the value of p), the closer we expect ${N_a}^*$ to approach to ${N_m}^*$, as fewer coerced copulations will need to be compensated for. On the other hand, where resistance is more costly, we expect to see the switch point occurring later, allowing the number of copulations to “overshoot” the female optimum slightly, as the cost of this will be balanced by lower resistance costs.

Table 1: *Details of the four mutually exclusive and exhaustive cases predicted by this model. Cases are numbered 1 to 4 in order of increasing values of k and of* ${N_a}^*$*.*

	Definition	**Strategic outcome**	**Factors limiting female fitness payoff**
Case 1	$k \leq 0$	No value of ${N_a}^*$. Female always accepts because resistance is too costly to be worthwhile.	Cost of excess copulations
Case 2	$0 < k < e^{-NB}$	${N_a}^* > N$. Female always accepts because she receives insufficient	Insufficient copulations

		copulation attempts to reach optimum number of copulations.	
Case 3	$e^{-NB} \leq k \leq e^{-qNB}$	Female begins resistance once $N_a{}^*$ is reached, compensating for expected number of forced copulations.	Cost of resistance balanced against cost of excess copulations to generate smallest possible total cost
Case 4	$k > e^{-qNB}$	$N_a{}^* < 0$. Female always resists because forced copulations alone will reliably constitute optimum copulation number (and possibly exceed it).	Cost of resistance and cost of excess copulations

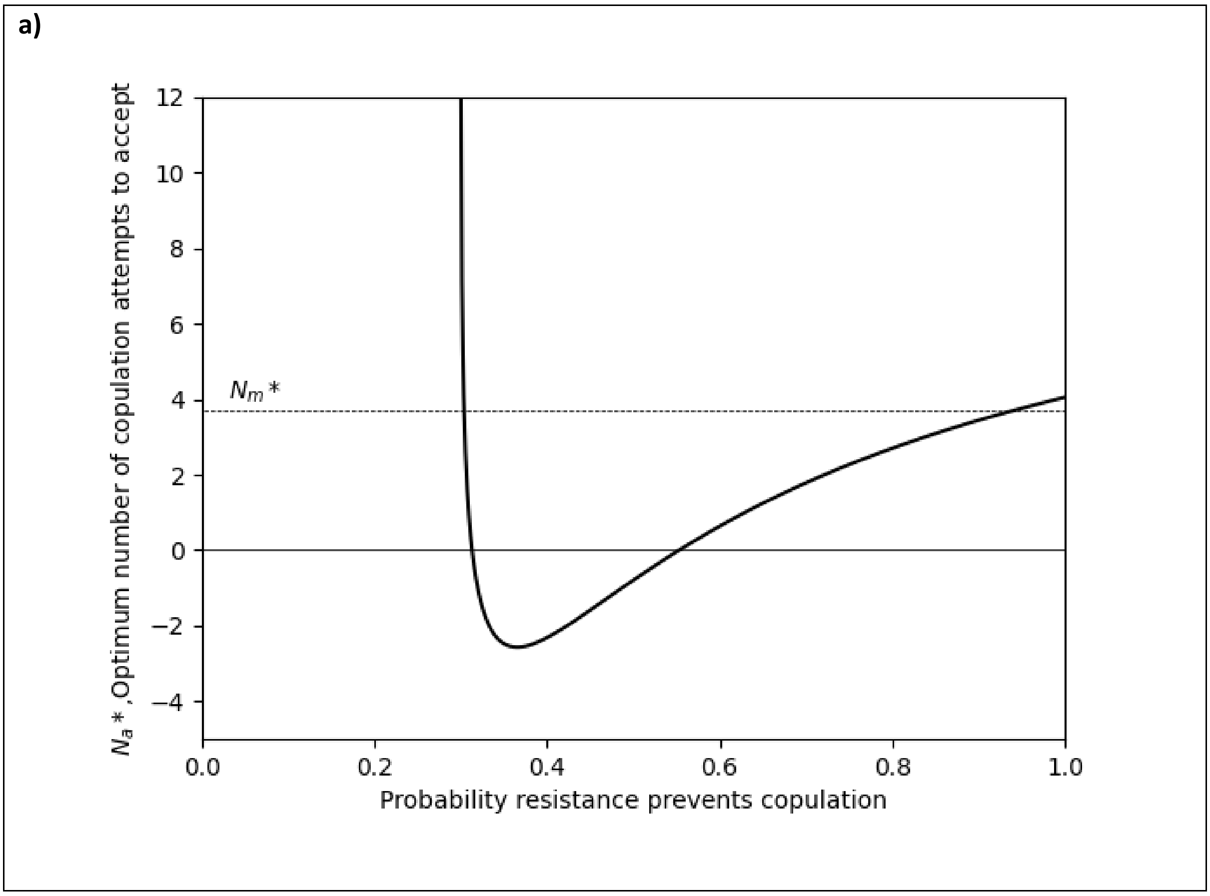
We expect case 4 to occur in situations where females expect many forced copulations to occur despite resistance, but resisting is cheap and effective enough to still be worthwhile. This may occur in situations where sexual harassment is high, interpreted in this model as high values of *N*, for instance due to male-biased sex ratios, or due to highly sexually dimorphic costs and benefits of copulation meaning that the optimum rate for males is much higher than for females. To fully compensate for the expected quantity of forced copulations and prevent the number of copulations from overshooting $N_m{}^*$, a negative value of N_a would be required (the mathematical solution to equation **5**). This is of course not possible, so the female's optimum strategy would "make the best of a bad job" by setting N_a as low as possible, at zero.

How does the predicted level of resistance vary?

We next set out to understand how levels of resistance may vary in nature given variation in the intensity of sexual harassment as well as the effectiveness and costs of female resistance. ***Figure 5***, ***Figure 6***, and ***Figure 7*** show how, while keeping the optimal number of copulations ($N_m{}^*$) fixed, the optimum behaviour of a female can vary widely with different values of effectiveness (p), resistance costs (r), and number of attempts expected (N). Unless otherwise stated, *A*=20, *B*=1, *C*=0.5, *p*=0.9, *r*=0.15, *N*=10. These values of *A*, *B*, and *C* give an $N_m{}^*$ of 3.67 using equation **2**. Each of these figures therefore describes a situation where the female's optimum number of copulations is 3.67 (out of 10 copulation attempts). However, varying the cost and effectiveness of resistance, and the number of copulation attempts expected, greatly alters the female's optimal behavioural strategy with regard to achieving this goal.

Effectiveness of resistance

We first assessed the implications of varying the effectiveness of female resistance (i.e. p; ***Figure 5***). Since the boundary to case 1 (convenience polyandry) occurs where $r = Cp$ (see **Cases**), this translates to where $p = r/C$, which in this case is when p equals 0.3. That is, when p, the effectiveness of resistance, is sufficiently low that the expected cost saved by resisting (cost of copulation, C, multiplied by the probability that resistance prevents copulation, p) is lower than the fixed cost of resistance, it no longer pays to attempt to control the number of copulations via resistance. In this area there is no solution to equation **5** and no value of $N_a{}^*$; that is, no point where a female benefits by switching from non-resistance to resistance.



b)

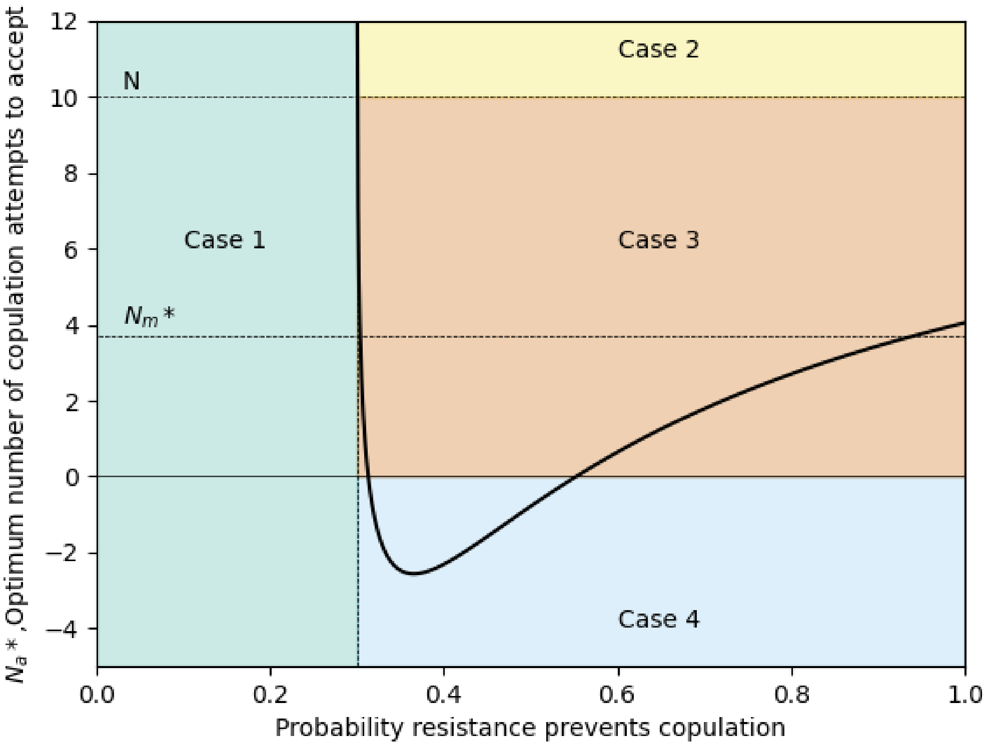


Figure 5: *Optimum number of copulations to accept (solid line) plotted against the effectiveness of resistance. The dotted line labelled $N_m{}^*$ shows the optimum number of copulations (= 3.67) from the female's point of view, given A = 20, B = 1, C = 0.5. The distance of the graph below this represents the optimum level of "compensatory resistance" (resistance that occurs before the optimum number of copulations has occurred). For clarity, the model predictions are first presented alone in **Figure 5a**, and the areas representing different cases are then illustrated in **Figure 5b**.*

For lower values of p, the value of $N_a{}^*$ moves further below $N_m{}^*$, as less effective resistance means more forced copulations are expected and so more compensatory resistance is required to achieve the same number of copulations. When $N_a{}^*$ drops below zero, case 4 is entered and we expect to observe the female accepting zero copulations, since it is not possible to accept a negative number of copulations.

When p is very close to this boundary (in the section just to the right of the vertical dotted line in ***Figure 5b***), $N_a{}^*$ asymptotically approaches infinity. This may be a point where the real-world applicability of the model becomes compromised. This asymptotic increase is a mathematical artefact of the logarithm term dominating as k descends towards 0. In reality, as p decreases, we might expect a female to switch straight from case 4 (always resist because resistance so ineffective that it needs to be “on” all the time) to case 1 (always accept because resistance is even less effective, and no longer worth the cost) without passing back through case 3 and then 2, as this graph implies.

For intermediate values of p, around 0.3 to 0.6, the female is expected to show behaviour described in case 4. Theoretically, the maximum possible payoff would be given by accepting a negative number of copulations (graph line is below the x axis, revealing that ${N_a}^* < 0$). In this situation we expect females to resist all attempts at copulation: resistance is not very effective, so it needs to be used to the maximum to get as close as possible to the optimum result. When p increases more, however, the graph rises above the x axis and we enter case 3. This is the point where resistance is sufficiently effective that having it “on” all the time might lead to insufficient copulations. However, since ${N_a}^*$ is still lower than ${N_m}^*$ (indicated by the labelled horizontal dashed line), the female is still engaging in compensatory resistance, accepting fewer copulations than her optimum number to actually occur.

At very high values of p, around $p \geq 0.9$, the graph line rises above ${N_m}^*$. This implies that females should not become resistant until *after* their optimum number of copulations have already occurred (the opposite of compensatory resistance). This is because the cost of resistance means the optimal strategy is to accept a number of copulations that will result in a slight “overshoot” of ${N_m}^*$, as the cost of excess copulations will be balanced by reduced resistance costs associated with higher values of N_a. If r was set to zero, then at $p = 1$ the optimum number of acceptances would be exactly equal to the optimum number of copulations, because with a perfectly cost-free and effective method of preventing copulations from occurring there is no need for a female to engage in compensatory resistance. Instead, she can begin resistance when the optimum number of copulations has been reached.

Except for the asymptotic increase in ${N_a}^*$ near the point where $p = r/C$, we do not enter case 2 in this graph. This is because with the values set for *A*, *B*, *C*, and *N*, the female’s optimum number of copulations ${N_m}^*$ is quite far below *N*, the number of attempts expected. These quantities are independent of p, so there is no value of p where this is not the case. We would interpret this to mean that sexual harassment is quite high, perhaps because the optimum copulation rate is much higher for males than females, or because of a male-biased sex ratio. For ${N_a}^*$, the optimum number to accept, to be above *N* (the defining characteristic of case 2) would require ${N_a}^*$ to be almost three times higher than ${N_m}^*$. Biologically, this would be a very unexpected outcome: the only reason you would expect a female to benefit by accepting so many copulations in excess of her optimum would be if there were extremely high resistance costs - but at this point, resistance would likely be so costly that it would never be worthwhile (case 1). If *N* is closer to ${N_m}^*$, or below it, then there is a wider range of values of p and r that are expected to produce case 2 behaviour (see ***Figure 7***).

Cost of resistance

We next assess the implications for behaviours as a function of varying costs of resistance (***Figure 6***). Plotting the optimum number of accepted copulations, $N_a{}^*$, against the cost of resistance, r, demonstrates the trade-off between resisting attempts in order to limit the costs associated with copulations, and accepting attempts in order to avoid engaging in costly resistance.

At the point where $r = 0$, (left hand side of the graph), the optimum number of copulation attempts to accept, $N_a{}^*$, will result exactly in $N_m{}^*$, the optimum number of copulations (the number of copulations being the sum of the number of attempts accepted and the number unsuccessfully resisted). This is because if resistance is cost-free there is no reason not to resist exactly as much as is required to achieve the optimum number of copulations (provided that resistance effectiveness is greater than zero). The distance of the graph line denoting $N_a{}^*$ below the dashed line denoting $N_m{}^*$ at this point shows that compensatory resistance is needed to achieve this number of copulations: females should begin resistance before $N_m{}^*$ copulations have occurred, because this number is expected to be reached through $N_a{}^*$ accepted copulations and ($N_m{}^*$ - $N_a{}^*$) unsuccessfully resisted copulations.

As the cost of resistance increases from zero, $N_a{}^*$ also increases. Crucially, the optimum number of copulations $N_m{}^*$ has not changed, and neither has the effectiveness of resistance. This shows that females should optimally accept an increasing number of costly excess copulations, with the optimal level of convenience polyandry increasing with increasing resistance costs. At the point where $r = Cp$, case 1 is entered, and it is not beneficial for a female to engage in any resistance, no matter how many copulations result. As in ***Figure 5***, we see the graph line asymptotically approach infinity as it approaches this boundary, beyond which there is no value of $N_a{}^*$. Again, this asymptotic approach is a mathematical artefact and so results should be interpreted with caution. However, the predicted pattern of steady increase in $N_a{}^*$ with increasing r, followed by a rapid increase tending towards infinitely high $N_a{}^*$ (i.e. case 1) as r approaches Cp, may be biologically plausible.

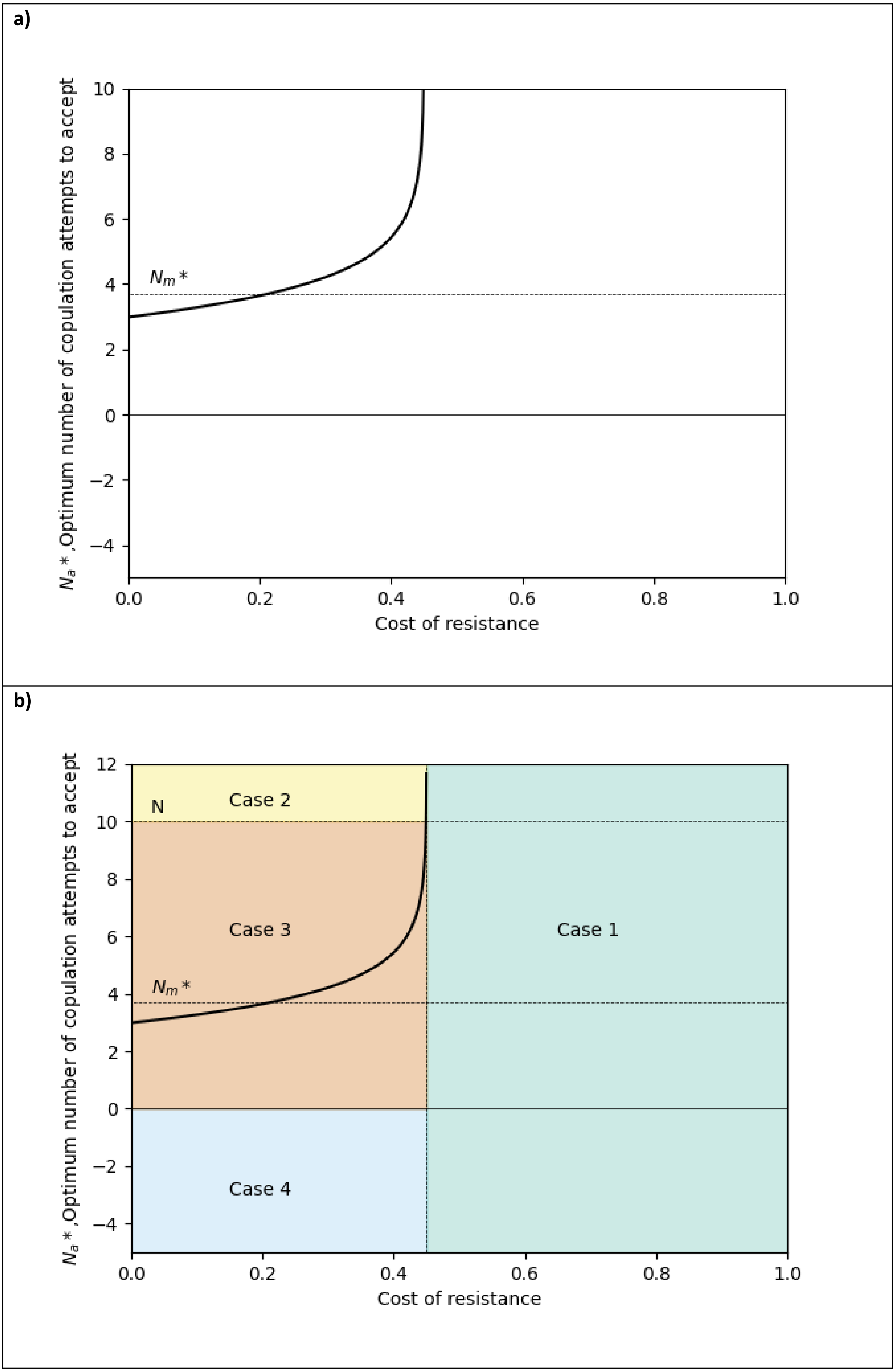

a)
N_a*, Optimum number of copulation attempts to accept
10
8
6
4
2
0
−2
−4
N_m*
0.0
0.2
0.4
0.6
0.8
1.0
Cost of resistance
b)
N_a*, Optimum number of copulation attempts to accept
12
10
8
6
4
2
0
−2
−4
N
Case 2
Case 3
Case 1
N_m*
Case 4
0.0
0.2
0.4
0.6
0.8
1.0
Cost of resistance

Figure 6: *Optimum number of copulation attempts to accept plotted against cost of resistance. The dotted line labelled* $N_m{}^*$ *shows the optimum number of copulations (= 3.67) from the female's point of view, given A = 20, B = 1, C = 0.5. For clarity, the model predictions are first presented alone in* ***Figure 6a****, and the areas representing different cases are then illustrated in* ***Figure 6b****.*

With increasing costs of resistance, females benefit by resisting less, accepting more attempts, even though their optimum copulation number does not change. As r approaches the boundary given by r = Cp, indicated by the vertical dashed line in ***Figure 6b****, the optimum number of attempts to accept sharply increases. This is because beyond this point resistance can never be worthwhile, as the fitness costs are greater than the maximum possible cost saved by not copulating.*

Number of attempts expected

Finally, we assess the relationship between the expected number of copulation attempts a female will receive and her optimal number of attempts to accept (***Figure 7***, next page). This shows that we expect a linear decline in acceptance rate as *N* increases. The higher the value of *N*, the higher the number of forced copulations females should expect and account for with compensatory resistance. On the other hand, higher values of *N* imply higher resistance costs for the same value of N_a, which increases $N_a{}^*$, making the slope shallower than it would be if resistance costs were zero.

Case 2 (always accept attempts) is reached when $N_a{}^*$ is greater than N, i.e. when the graph line passes into the region above the diagonal dotted line for low values of *N* in ***Figure 7b***; this indicates that the number of attempts females expect to receive is smaller than their optimum number to accept. Case 4 (always resist) would be reached when the graph dips below the x axis - this does not appear on this graph because the range of *N* shown is not large enough.

Case 1 (no solution) does not appear on this graph, because the boundary to case 1 occurs where $r = Cp$: it is not defined by N, the number of copulation attempts. This means that the model cannot switch in or out of case 1 if N is the only variable that changes. Either it is sometimes worthwhile to resist, or it is never worthwhile to resist, and this does not depend on the number of attempts that are expected.

By contrast, the upper and lower bounds of case 3 occur at $k = e^{-qNB}$ and $k = e^{-NB}$ respectively, meaning the boundaries are directly influenced by N and there will always be values of N where the model predicts case 2, case 3 and case 4 behaviour, whichever values the other parameters may take.

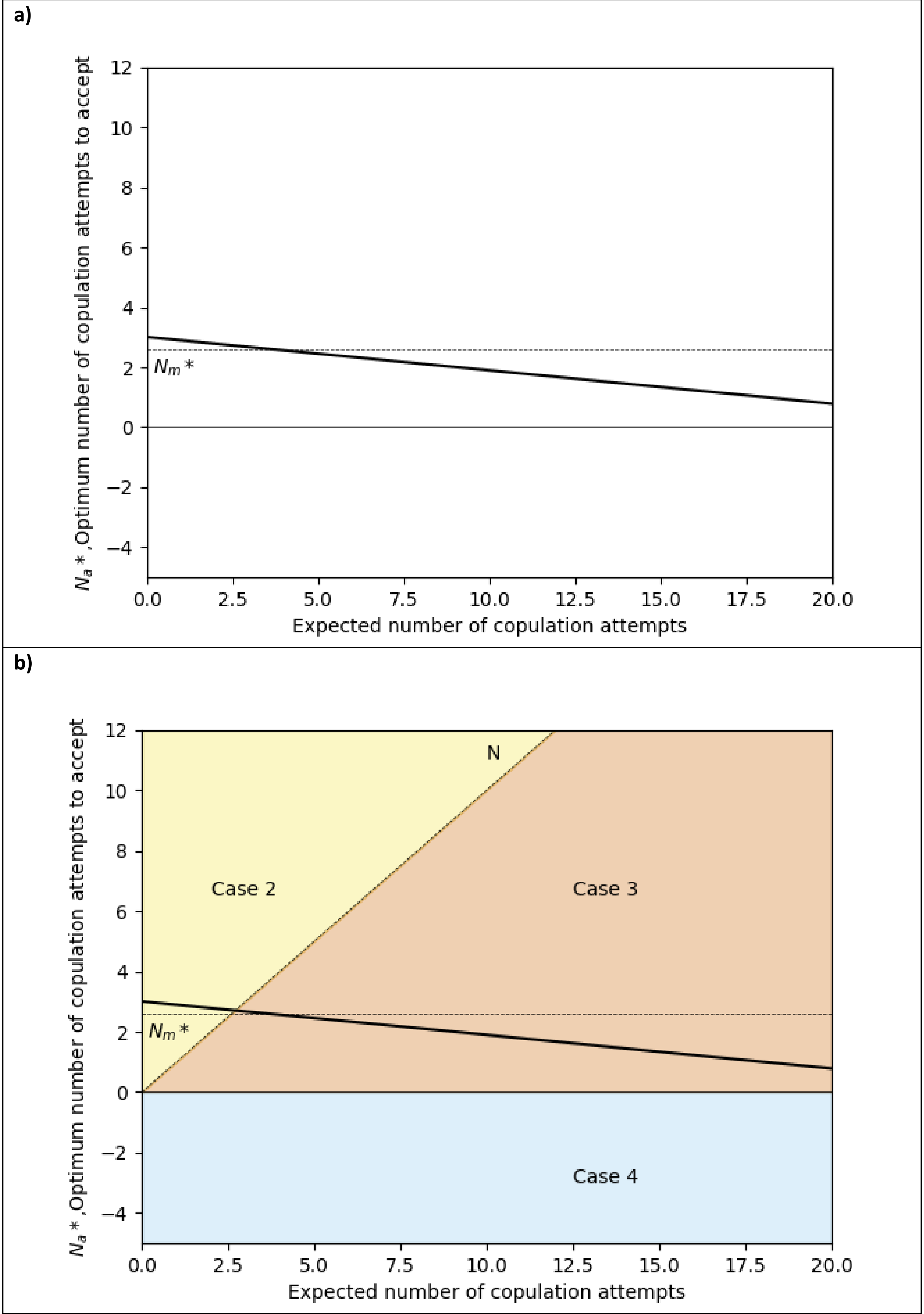


Figure 7: *As the expected number of copulation attempts increases, the optimum number to accept decreases, since there are more chances for forced copulations to occur, and so more resistance is*

*needed to maintain the same number of copulations. As before, the dotted horizontal line represents $N_m{}^*$, and the distance of the graph below this line represents the level of compensatory resistance. For clarity, the model predictions are first presented alone in **Figure 7a**, and the areas representing different cases are then illustrated in **Figure 7b**.*

Implications for empirical observations

Behavioural observations are likely to provide estimates of N, the number of copulation attempts females commonly receive; N_a, female receptivity, expressed as a proportion of attempts accepted; and p, the effectiveness of resistance. Estimating A, B, C and r, constants referring to the fitness costs and benefits of copulation and of resistance, is likely to be much more difficult.

If females either always resist or always accept copulation attempts, then this could be described by case 4 or by case 1 or 2 respectively. In these cases, we would not be able to estimate $N_a{}^*$ from behaviour. There are infinitely many theoretical values of $N_a{}^*$ below 0 that would map to a real-world behavioural strategy of accepting 0 copulation attempts, and likewise for values above N. It is therefore impossible to work backwards from the observed values of N_a to determine $N_a{}^*$.

By contrast, any situation where females employ a strategy other than “always accept” or “always resist”, where females are sometimes receptive and sometimes resistant, could be said to be described by case 3 of this model. If females behave adaptively, then the observed value of N_a could be said to reflect $N_a{}^*$, the number of acceptances expected to produce the highest fitness payoff, taking the expected effects of sexual coercion into account.

Because resistance has a cost r, the optimal value of N_a ($N_a{}^*$) will not be the one which would result in the optimum number of copulations, $N_m{}^*$. Instead, we expect $N_a{}^*$ to “overshoot” $N_m{}^*$, by some amount we will call w (see ***Figure 8***). The cost of exceeding the optimum number of copulations is offset by reduced costs of resistance at $N_a{}^*$, compared to the value of N_a which would have resulted in $N_m{}^*$ copulations.

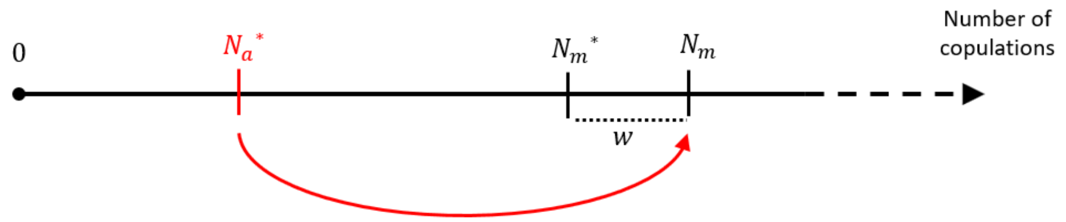


Figure 8: *Number of copulations is represented on a number line increasing from 0. Each value of* N_a *(number accepted) maps to a resulting value of* N_m *(number occurring), which is made up of the number accepted plus the number unsuccessfully resisted. The optimum value of* N_a*,* ($N_a{}^*$*, marked in red) is one that will overshoot* $N_m{}^*$*(the optimum value of* N_m*) by an amount dependent on the cost of resisting. The amount of overshoot is labelled* w.

From this it is possible to derive an expression for $N_m{}^*$ given an observation of $N_a{}^*$, subtracting the value of w (represented in the final term of equation **7**) from the N_m that results from $N_a{}^*$ (see Appendix to this chapter). When $r = 0$, i.e. resistance is cost free, the term representing w is also zero: there is no overshoot, and $N_a{}^*$ maps directly to $N_m{}^*$.

$$\mathbf{7} \qquad N_m{}^* = pN_a{}^* + qN - \frac{1}{B}\ln\left(\frac{C}{C-\frac{r}{p}}\right)$$

This equation makes the prediction that, if we are in case 3 and females are behaving adaptively, then $N_m{}^*$, the female's optimum number of copulations, should be lower by w than the number of copulations observed, an amount defined by the costliness of resistance.

It also makes the prediction that it is possible for a female to "win" sexual conflict over copulation (achieving $N_m{}^*$, her optimum copulation number) despite experiencing forced copulations, by adjusting receptivity downwards to take forced copulations into account. Crucially, since departure from $N_m{}^*$ is taken to be strategic "overshooting" on the part of the female, accounting for costs of resistance, if resistance is cost-free then a female's chosen strategy is expected to result in her optimum number of copulations. If a female copulates more than her optimum number of times, while in case 3 of this model, then this departure from her optimum is due to the costliness of resistance, not because she physically could not have prevented excess copulations from occurring: a subtle difference in perspective.

Discussion

Female resistance to copulation is increasingly recognised as an important factor influencing the operation of sexual selection (Arnqvist and Rowe 2002; Parker 2006; Perry and Rowe 2018; Rowe et al. 1994). However, the characterisation of patterns of resistance – how resistance varies both on evolutionary timescales and plastically within an individual's lifetime – remains in its infancy.

The model described here provides a framework through which to interpret female resistance to copulation as a key variable within the study of sexual selection and to clarify the underlying selection pressures that give rise to it. This model incorporates the effectiveness of resistance as well as its cost to determine the optimal point for females to begin resistance, and finds that varying the cost or the effectiveness of resistance, or the level of sexual harassment expected, can give rise to very different optimal behaviours for females even when the optimal copulation rate remains fixed. The idea that females ought to plastically alter resistance behaviours in response to differing costs is supported by existing theoretical and empirical work (McLean and Stuart-Fox 2010), as is the idea that resistance should increase in response to increased harassment (Kazancioğlu and Alonzo 2012; McLean and Stuart-Fox 2010). However, existing models of female resistance behaviour do not explicitly consider that the incomplete effectiveness of resistance predicts "compensatory" resistance (resistance of copulation attempts that would be of fitness benefit to a female) to account for an expected number of forced copulations that are unsuccessfully resisted.

If a female is resistant to copulation, this has two possible evolutionary explanations. Firstly, that further copulations would be more costly than beneficial, and therefore her fitness is best served by avoiding them— this might be termed 'true' resistance, in the sense that female fitness is maximised if this resistance is successful in preventing copulation. The second explanation is that further copulations would be beneficial in terms of fitness, but due to sexual coercion, copulation is likely to occur even if she resists. Resistance in this second situation is here termed compensatory resistance. Unlike 'true' resistance, female fitness would not be maximised by this resistance preventing copulation; rather, females engage in this resistance to compensate for the fact that resistance is not totally effective. These two explanations can coexist: where females are resistant to copulation, some portion of resistance will often be attributable to compensation for sexual coercion, while the remainder could be considered 'true' resistance. This model attempts to differentiate between these two motivations for resistance in order to better understand the underlying evolutionary pressures shaping sexual strategies.

Central to this model is the distinction between ultimate and proximate explanations for behaviour. Evolutionary pressures provide the ultimate explanation for many behaviours and traits, which act via individuals' psychological mechanisms as the proximate motivator for behaviours (Dewsbury 1999). Thus, compensatory resistance does not imply that a female is attempting to "trick" a male, or is only putting up a show of resistance. The ultimate evolutionary goal of achieving a certain number of copulations is expected to select for the appropriate copulation propensity that is most likely to produce optimal outcomes, while incurring the fewest additional costs, in a given environment. For instance, in case 4 of this model (always resist), females might be selected to have an extreme aversion to males, as females with this aversion might incur the lowest costs associated with excess copulation. This total aversion would not imply that a female's fitness is ultimately maximised by never copulating. Instead, it implies that some copulations occur despite resistance, and the proximate behavioural mechanism of total resistance produces the optimal outcome for males because it prevents copulation rates from rising to excessively high levels.

Likewise, because this model incorporates a cost of resistance, we can differentiate acceptance of copulations into two distinct types based on ultimate motivation. Firstly, what we could call "true" acceptances: the case where females accept copulations because they are beneficial to their fitness. Secondly, convenience polyandry: the case where copulations are not beneficial to female fitness, but accepting surplus copulations has a lower cost than resisting them. This second case is likely to be very common within the animal kingdom, as the imposition of resistance costs in the form of persistent harassment is a common tactic of sexual coercion on the part of males (Clutton-Brock and Parker 1995). This is the permanent condition for individuals in case 1, where the costs of resistance are so high relative to the expected payoffs that there is never a point where it is optimal to begin resistance. An element of convenience polyandry is also seen in cases 2, 3, and 4, where ${N_a}^*$, the optimal point to begin resistance, is one that gives a value of N_m consistently higher than the optimum, as the cost of 'overshooting' the optimum copulation number is balanced by reduced resistance costs.

The optimum number of copulations for individuals of both sexes, and especially for females, is a key variable within the study of sexual conflict, and has been the subject of theoretical and empirical work for decades (Arnqvist and Nilsson 2000; Jennions and Petrie 2007; Ridley 1990; Slatyer et al. 2012). It is not expected that the model detailed here will be most useful in making quantitative predictions as to females' optimum copulation number, due to the extreme difficulty in estimating many of the model's parameters. It is also not easy to estimate parameters based on existing literature, because little empirical work exists that treats female resistance in detail as a main dependent variable, though some detailed studies of the sexual behaviour of particular species provide indirect estimates for both the receptivity of females (i.e proportion of copulation attempts accepted) and the effectiveness of their resistance in preventing copulation from occurring (e.g. Mitani, 1985).

Rather than making detailed quantitative predictions based on empirical data, the primary intention for this model is to contribute a useful conceptual framework for interpreting female resistance behaviour. We explicitly consider N_a, the number of copulations accepted; N_m, the number of copulations that occur; and ${N_m}^*$, the female's optimum number of copulations, and set out mathematically how these values may relate to each other. These can be used to make broader predictions as to the expected impact of altering ecological parameters. For instance, if females' ability to resist is decreased, the female's optimal outcome (${N_m}^*$) is not expected to change, but the level of "compensatory resistance" required to achieve the same number of copulations will increase. We would therefore expect to see a decrease in the receptivity of females; crucially, however, it would be incorrect to interpret this decrease in receptivity as signifying a lower optimum copulation rate for the female (for instance see Rowe 1992).

This same principle also could be used to supply alternative hypotheses in cases where females exhibit more resistance to some males than to others. For example, female mate choice favouring larger males is widespread (Friberg and Arnqvist 2003; Wilkinson, Kahler, and Baker 1990). In Mitani's (1985) study into sexual behaviour among wild orangutans, females were found to be much more receptive to copulation attempts from males of the large morph (46% of copulations were forced), compared to males of the small morph (95% forced).

It is tempting to interpret this discrepancy as resulting from a female preference for larger males (in the terms of our model, a higher ${N_m}^*$ for large males than small), but this model suggests alternative hypotheses based on proximate factors. From equation **5** and ***Figures 5, 6*** and ***7***, we can see that a reduction in ${N_a}^*$, the female's strategic optimum in receptivity, could also theoretically be brought about by a lower value of p or N or a higher value of r- less effective resistance, costlier resistance, more expected copulation attempts to be fended off; or some combination of the three. Variation in male size is likely to be associated with variation in these parameters (*p, N*, and *r*) (Friberg and Arnqvist 2003; Kim et al. 2021; Ortigosa and Rowe 2003). We should therefore expect some adaptive variation in the receptivity shown by females to males of different sizes that is *not* explained ultimately by female preference for one phenotype over another.

Similarly, in species with a strong social hierarchy, it is very plausible that resistance could be less costly and more effective against subdominant males, since dominant males often interrupt copulation attempts by their social inferiors, and can sometimes be recruited to do so by females (Løvlie, Zidar, and Berneheim 2014). Females have no such recourse against sexual harassment by dominant males. Dominant males may also make more copulation attempts, due to their ability to aggressively exclude subordinates (McDonald et al. 2017). This raises the question of to what extent differing *p, r,* and *N* could explain the widely reported preference of females for dominant males, which may imply lower preference via good genes, sexy sons, and other explanations for female sexual preferences.

It is clear that considering only ultimate explanations (i.e., assuming that greater receptivity towards certain males implies that females benefit more in terms of fitness by copulating with those males rather than others) risks overlooking other explanations for differential receptivity based on proximate factors. It is possible that a female could derive identical fitness benefits from copulating with two different males, but require different levels of compensatory resistance- and hence, different observed behaviour- with each to achieve the same end.

This model makes a number of simplifying assumptions. For instance, female resistance strategies are treated in the simplest possible way: females have two states, non-resistant and resistant, and they can make a permanent switch from one state to the other, once, and in one direction only. In reality, however, courtship and copulation are often a complex series of behaviours with several decision points where resistance may be introduced, with each with different associated costs and effectiveness (McLean and Stuart-Fox 2010; Sparkes, Keogh, and Haskins 2000). In many cases, female resistance has the effect of terminating copulation and limiting the quantity of ejaculate transferred from the male, rather than simply preventing copulations from occurring (eg Crudgington and Siva-Jothy 2000; Friesen et al. 2013); this is a dimension of resistance which has not been accounted for in this model. For many species, more sperm are transferred the longer copulation continues, with consequent higher chance of fertilisation (Eberhard 1985). Some costs, such as energetic costs and increased predation risk, are likely to scale upwards with time spent copulating. We therefore expect sexual conflict over copulation to encompass both conflict over whether copulation occurs, in which males attempt to copulate and females resist; and conflict over its duration, in which females attempt to terminate copulations and males attempt to continue. This model could be adapted to focus on these effects by making resistance a continuous variable with its own functions of cost and effectiveness at different levels, rather than a simple binary of “on” or “off”.

In addition, this model treats all individuals and all encounters identically. In reality, individuals within a species are likely to vary very significantly in terms of the parameters that generate this model’s predictions, both due to their phenotype and their ecological setting (García-Roa et al. 2020; Perry and Rowe 2018). For instance, more attractive females are likely to be subject to significantly greater intensity of sexual harassment (Long et al. 2009), increasing their value of N and consequently decreasing their value of ${N_a}^*$ relative to less attractive females, even if the optimum number of copulations were identical for every female. Reinhardt, Naylor, and Siva-Jothy (2009) report that in the bedbug *Cimex lectularius* female resistance to copulation is significantly reduced after feeding, but that this reduction in resistance is likely due to physiological constraints, since the female’s size increases by around 300% after a blood meal, precluding normal resistance behaviours (and presumably lowering *p*, the probability that they are able to successfully resist).

Further complexity is added by the fact that individual variation in one model parameter is likely to be non-independent of variance in other model parameters. For instance, in their model of the coevolution of female resistance and male persistence traits, Kazancioğlu and Alonzo (2012) argue that costs of copulation in terms of direct harms to the female are likely to covary with male persistence traits; males that are more aggressive may both be more persistent and more harmful to females, a factor that made a significant difference to their model’s predictions. In terms of the model this would mean that males against whom females have lower effectiveness of resistance *p* are likely to have both greater copulation costs *C* and greater resistance costs *r*. We have already seen how males that may be more attractive to females in several respects (larger size and more socially dominant) may have lower associated p and higher r. In addition, in general, males who are better at overcoming resistance (i.e. against whom females have lower p) are also expected to be more attractive to females (greater $N_m{}^*$) due to "sexy sons" effects (Eberhard 1985; Gavrilets, Arnqvist, and Friberg 2001).

These factors add up to a structured network of interactions that is vastly more complex than this model accounts for. Our model is based on a simplifying assumption that interactions take place in the context of an infinitely mixed population where any individual of the opposite sex is equivalent as a potential sexual partner to any other. However, real individuals are concerned with copulating with suitable partners (high genetic quality, avoiding incest and incompatibility), rather than simply copulating indiscriminately until a quota is reached. Spatial structure means that repeated encounters between the same individuals are likely in many real-world scenarios. Parameter values are likely to be different not just for every individual but for every possible pair interaction. It is not feasible to include every possible complication in a model. However, depending on the focus of future research questions, it may be possible to update this model to consider different population subsets, or even each individual, as having individualised parameter values; or to allow the structure of social networks to determine the rates of encounters in an agent-based simulation.

This chapter shows how optimal levels of female resistance to copulation depend not only on the optimal number of copulations for females, but also on proximate ecological factors. In particular, it demonstrates that the incomplete effectiveness is expected to have predictable effects on optimal female strategies, including the promotion of "compensatory resistance". Compensatory resistance is a kind of mirror image of convenience polyandry. Just as convenience polyandry (i.e. copulation is not a female's optimal outcome) is often considered as a potential explanation for the acceptance of multiple copulation attempts by females, compensatory resistance (i.e. the prevention of copulations is not a female's optimal outcome) ought to be carefully considered as a potential explanation for observations of female resistance to copulation.

Chapter 2: Effects of sexual novelty on sexual behaviour of female fowl

“The President and Mrs. Coolidge were being shown around an experimental government farm. When she came to the chicken yard she noticed that a rooster was mating very frequently. She asked the attendant how often that happened and was told, ‘Dozens of times each day.’ Mrs. Coolidge said, ‘Tell that to the President when he comes by.’ Upon being told, Coolidge asked, ‘Same hen every time?’ The reply was, ‘Oh no, Mr. President, a different hen every time.’ Coolidge: ‘Tell that to Mrs. Coolidge!’”

- Frank A. Beach, quoted in Kimble, Garmezy and Zigler, *Principles of General Psychology, 4th ed.* (1974)

Abstract

Novelty modulates sexual motivation in many species, including fowl. The potential for increased number of offspring means that male fowl prioritise copulation opportunities with novel females, and show decreased propensity to mate with familiar females, as well as decreased investment in ejaculates - a phenomenon known as the Coolidge effect. Females cannot gain offspring through multiple sexual partners in the same way, but may still derive other benefits from the prioritisation of unfamiliar mates, such as increased genetic diversity of offspring. Female copulation propensity is difficult to study, however, and the effect of novelty remains less well characterised than for males. Fowl represent an especially interesting system in which to study female responses to novelty, because female strategies must intersect with the male Coolidge effect (novel males are expected to provide greater sperm investment, for instance, but may also show more sexual harassment of females) and with a high degree of sexual coercion by males. We demonstrate for the first time that female copulation propensity in this species declines with familiar males, and that it may be revived by the presence of novel males (a Coolidge-type effect). These results provide detailed insight into how females exercise control in situations of sexual coercion. Females are able to influence copulation rate through selectively soliciting copulations, selectively resisting male-initiated copulations, and through patterns of space use (we provide empirical evidence backing the assumption that females use perching as a means of influencing copulation rate). Older females perch more than younger females, but other than this we do not detect any differences in strategies used by female age or social status.

Contributions

The chapter and experimental design were conceived by Tommaso Pizzari and significantly developed by Ellen Pasternack. Data was collected by Ellen Pasternack (50%), Lorena Boisseau (25%) and Anna Garcia (25%). Lorena and Anna were recruited as research assistants for this project by Tommaso Pizzari. Statistical analysis was conceived and carried out by Ellen Pasternack, with input from Eleanor Bath and Tommaso Pizzari. The chapter was written by Ellen Pasternack, with input from Eleanor Bath and Tommaso Pizzari.

Introduction

Effect of sexual novelty in males

All living things face high-stakes decisions and trade-offs over how to allocate reproductive resources. In sexually reproducing species, one of these decisions may be how much to invest in a currently available mate, as opposed to holding back resources to invest in potential future mates.

This problem and its solutions have been extensively studied in males. A male's reproductive success is a function of his summed fertilisation successes across all the females with whom he copulates (Trivers 1972). Thus, inseminating a second female has the potential to double a male's number of offspring, while the number of offspring that can be gained by repeated copulations with a single female is fundamentally limited by that female's fecundity. Therefore, directing courtship and copulation effort towards unfamiliar or sexually novel partners is likely to be adaptive if it results in spreading copulations across more females. Unsurprisingly, this is what we see males doing across many species (eg. Kelley and Magurran 1999; Pizzari et al. 2003; Schnell et al. 2015; Simcox et al. 2005; Tokarz 2006).

However, sperm and seminal fluid are a costly resource: they are physically depleted by copulation, and can take some time to be replenished (Dewsbury 1982; Parker and Pizzari 2010; Wedell, Gage, and Parker 2002). Males may be physically incapable of inseminating a female again after having done so previously (Preston et al. 2001), meaning judicious ejaculate expenditure is extremely important. Declining investment with each successive copulation is expected as an adaptive adjustment to smaller and smaller marginal gains in fertility from each successive copulation with the same female. As expected, the latency to each copulation, and the number of sperm in successive ejaculates, show exponential decay in many species including rodents (Beach and Jordan 1956; Beach and Rabedeau 1959; Larsson 1956), humans (Levin et al. 1986), and junglefowl (Pizzari et al. 2003).

These factors combined – declining investment over repeated copulations, plus preference for novel partners – can give rise to an effect where sexual interest dwindles with continuous access to one partner, but is revived when presented with a new partner. This phenomenon is dubbed the 'Coolidge Effect', for an anecdote recounted in the epigraph of this chapter involving President Calvin Coolidge and a libidinous chicken. It was first scientifically documented through experiments on rats in the 1960s (Fisher 1962; Fowler and Whalen 1961; Wilson, Kuehn, and Beach 1963), and since then has been described in a large number of different species and situations (Dewsbury 1981; Koene and Maat 2007; Spence, Reichard, and Smith 2013).

However, the Coolidge effect is not universally seen among males. For a male to show a Coolidge effect, he must refrain from copulating with one female, or hold back some sperm and seminal fluid during copulations, in case he later has the chance to copulate with another. If copulations with multiple females are rare, this strategy is unlikely to be adaptive, as it would often lead to the waste of this reserved portion of sperm. For instance, Gray and Dewsbury (1973, 1975) compared the responses of two species of vole, the meadow vole *Microtus pennsylvanicus* and the prairie vole *M. ochrogaster*. While the former showed a clear Coolidge effect, the latter, a strongly monogamous species, did not: males reached sexual exhaustion and were not re-excited by a new female. For this reason, Thomas and Birney (1979) suggest that the absence of a Coolidge effect may be an indicator of a monogamous mating system, though this has not been tested.

Effect of sexual novelty in females

While we can make informed predictions about how males might react to mate novelty under different social or ecological conditions, far less is known about the effects of partner sexual novelty in females. In contrast to males, who can in theory multiply their reproductive success as they mate with multiple partners, the reproductive success of females is expected to have a strict limit set by her own fecundity. The observation that females of many species seek out polyandrous copulations (copulations with multiple males) is therefore something of a mystery. Many potential fitness benefits of polyandry have been identified that could explain this behaviour in some instances, but there is little in the way of overarching framework (Parker and Birkhead 2013; Pizzari and Wedell 2013).

Exploring female responses to partner sexual novelty in different situations would greatly add to our understanding of the selection pressures acting on female sexual behaviour. For instance, adaptive explanations for polyandry by females may be split into those where females obtain benefits by copulating with multiple partners, and those where females obtain benefits from multiple copulations per se, regardless of whether they are with different males or the same male. In the former situation, females might be expected to show a preference for sexually novel males; in the latter, sexual novelty would not be expected to affect mate choice (Archer and Elgar 1999).

One reason for this knowledge asymmetry is that sexual behaviour is interactive, and in many species it is males that mostly lead these interactions, making female behaviour much more difficult to study in a standardised way. Sperm numbers and ejaculate volume provide an objective measure of male sexual investment, which cannot be replicated in females. In addition to these practical constraints, the clear potential benefits to males of multiple copulations mean that male responses to novelty have been a more obvious research target.

As in males, we would expect to see a Coolidge effect in females in situations where two conditions are satisfied. Firstly, the act of copulation must have some non-negligible cost to females, so that females benefit by limiting the number of copulations. Secondly, polyandry must be advantageous to females, so that females have greater reproductive success when their limited number of copulations are spread over multiple partners rather than limited to a single partner.

The first condition is certainly satisfied in many species, since copulation often has significant costs to females, distinct from costs of reproduction and distinct from per-partner costs. These costs include somatic damage, negative fitness effects of exposure to ejaculates, time and energy costs, and increased exposure to predation. Copulation has been found to decrease lifespan in multiple model species (Fowler and Partridge 1989; Garratt et al. 2020; Gems and Riddle 1996; Jigisha et al. 2020). Females are therefore expected to benefit by limiting their copulations.

As for the second condition, the fitness premium attached to finding novel mates is not expected to be as large for females as it is for males, as female reproductive success is limited by individual fecundity as determined by physiological constraints. However, there are still some selection pressures that can favour polyandry. Copulating with multiple males has multiple potential benefits to females, including, increased genetic diversity of offspring, sexy sons via sperm competition, infertility/incompatibility insurance, increased nuptial feeding, and confusing paternity to protect against infanticide by unrelated males (Hrdy 1977, 2000; Jennions and Petrie 2007; Newcomer, Zeh, and Zeh 1999). Several meta-analyses have attempted to quantify the fitness benefits of polyandry, typically detecting either no effect or a modest benefit to females in terms of offspring number or survival (e.g. Arnqvist and Nilsson 2000; Slatyer et al. 2012). However, polyandry can also come at a cost to females in terms of their own survival (Arnqvist and Nilsson 2000). Since polyandry has many potential costs and benefits, whether the net benefit of polyandry is strong enough to promote a preference for sexually novel males is likely to vary by species, depending on the magnitude of costs and benefits to females.

As well as independent benefits of polyandry, it has also been suggested that the male Coolidge effect itself may push females towards a Coolidge effect of their own (Pizzari 2002). If males invest less and less in each successive ejaculation with a given female, sperm-limited females may be forced to seek out novel males (i.e., males to whom they themselves are novel) in order to obtain the maximum number of sperm in the same number of copulations. Another chief benefit of copulating is that it allows females to avoid the costs of resisting. If novel males harass females more, due to the male Coolidge effect, then acquiescing to copulations with novel males will allow females to avoid more resistance costs than copulations with familiar males. Females might therefore benefit from selectively resisting sexually familiar males, and accepting copulations from novel males, which would lead to a Coolidge effect.

A female Coolidge effect has only been directly tested and identified in one species, the golden hamster *Mesocricetus auratus* (Lester and Gorzalka 1988; Lisk and Baron 1982). As with the early studies demonstrating Coolidge in male rats, females in this species reach “sexual satiety” with one mate (defined as a period of time elapsed where females show no sexual receptivity to male attempts; in males the criterion typically used is no attempts) before renewing receptivity when provided with a new mate.

However, there is evidence in several other species of females favouring novel males (without necessarily showing the cessation and resumption of sexual activity that defines a Coolidge effect). In the polyandrous pseudoscorpion *Cordylochernes scorpioides*, females discriminated against previous mates when remating (Zeh, Newcomer, and Zeh 1998). In guppies (*Poecilia reticulata*), females discriminated against previous mates, as well as new males who looked like previous mates (Eakley and Houde 2004) and males with whom they were visually familiarised (Hughes et al. 1999). Similar preferences in females for sexually novel mates have been identified in choice trials in two species of cricket (Bateman 1998; Ivy, Weddle, and Sakaluk 2005) and in hide beetles, *Dermestes maculatus* (Archer and Elgar 1999). On the other hand, Schnell et al. (2015) report no preference for novel males in female cuttlefish *Sepia aparma*.

The junglefowl study system

This chapter investigates female responses to mate novelty in a population of captive red junglefowl, *Gallus gallus*.

As expected for a polygynandrous species, male fowl show a strong Coolidge effect. An early study found that male feral fowl and junglefowl showed a Coolidge pattern in the propensity to copulate with a female, the likelihood of transferring sperm in each copulation, and in the number of sperm transferred (Pizzari et al. 2003). A more recent study found that not only the number of sperm per ejaculate but also the concentration of seminal fluid proteins showed a Coolidge response, decreasing sequentially with each copulation before increasing again when copulating with a novel female (Alvarez-Fernandez et al. 2019). Since male fowl are subject to intense sperm competition, we expect their ejaculate composition and expenditure, as well as their pre-copulatory behaviour, to be highly sophisticated and adaptive. Though it has not been directly tested, it is possible that females of this species could also show a Coolidge effect. The first condition under which we would expect to see a Coolidge effect – costs of copulation – is met. Veerus (2021) found that copulations disrupt the reproductive microbiome of female junglefowl, with potential negative fitness consequences (Rowe et al. 2020). Carleial, McDonald, and Pizzari (2020) showed that in female red junglefowl, exposure to male harassment and forced copulation attempts is associated with loss in body mass (though a loss associated with the actual number of copulations was not detected). In addition, female fowl often try to evade male attention and resist copulation attempts, and can be treated roughly or aggressively by males (Millman, Duncan, and Widowski 2000). It seems likely that it is often adaptive for female fowl to limit their number of copulations (not just their number of sexual partners). Experimental evidence for this was found by Løvlie, Cornwallis, and Pizzari (2005), who report that in junglefowl, female resistance increases after being mated or mounted by males, indicating that females may attempt to limit their number of copulations (and perhaps that mounting is a heuristic used to accomplish this).

The second condition for a Coolidge effect – polyandry conveying a net fitness benefit to females – may also be met in this species. Many researchers have argued that polyandry serves as insurance for females, guarding against incompatibility or infertility of any one particular male (Birkhead and Møller 1992). In the fowl, we know that many copulations or copulation attempts do not result in sperm transfer (Assersohn, Brekke, and Hemmings 2021; Løvlie and Cornwallis 2005). Females store sperm internally for up to a month, but fertility declines with longer-stored sperm (Pizzari et al. 2007; Wishart 1987). Laying females need to fertilise one ovum daily over a succession of days; therefore, the need to maintain internal stores of fresh, high-quality sperm may motivate repeated copulations. Since there is large between-male variation in fertilising ability and sperm quality (Birkhead et al. 1999; Robertson et al. 1998; Wilson et al. 1979), this may mean ensuring fertilisation is a significant motivator of polyandry in this species. In addition, costs of resistance are likely to be high in this species (Carleial, McDonald, and Pizzari 2020; Løvlie and Pizzari 2007). Since males are more motivated to mate with novel females, it may be more costly for females to resist novel males (i.e. males to whom they themselves are novel), as this may involve a more protracted or aggressive struggle. This could lead females to selectively resist familiar males as a form of convenience polyandry.

As a counter, however, some costs of copulation may increase with the number of partners, providing pressure in the opposite direction. For instance, the risk of contracting a sexually transmitted infection likely scales up more per partner than per copulation event. This risk is likely high in this species, due to its polygynandrous mating system, and due to the mechanism of ejaculate transfer via the cloaca, which means contamination with gut flora is likely. Infections transmitted via ejaculates can reduce female fertility in fowl (Haines et al. 2015) and promote dysbiosis of the reproductive tract (Veerus 2021). The male Coolidge effect, whereby males show greater motivation to mate with sexually novel females, may also mean that copulations with novel males are associated with greater stress and risk of somatic damage for females – see, for example, water striders, where females in tandem pairs (i.e. paired with a 'familiar' male) forage more efficiently than single females who are continually harassed by novel males (Wilcox 1984). These factors may promote an 'anti-Coolidge' effect, whereby females show greater receptivity to sexually familiar males in order to minimise costs associated with novel males.

The polygynandrous mating system of this species means that almost all females mate multiply (Pizzari and McDonald 2019). A free choice trial by Ligon and Zwartjes (1995) reports that almost all females chose to mate with both males offered, suggesting that female as well as male preferences may contribute to the high level of polyandry in junglefowl. However, since a high proportion of copulations in this species are forced, it is difficult to elucidate the level of female preference for polyandry. Females may be coerced into polyandrous copulations that are not adaptive for them. Additionally, polyandrous copulations may be adaptive for females without females necessarily needing to show any proactive behavioural preference for polyandry, due to the fact that polyandrous copulations is likely to occur anyway, driven by male strategies. This would be a form of 'compensatory resistance' as discussed in chapter 1.

Copulations in fowl result from a complex interplay of male and female behaviours, and can take place in several different ways, each of which is under different levels of female control (see ***Table 3***). Females can influence the outcome of these interactions at several stages. Female choice of social associations has a significant impact on whether and with whom they copulate (McDonald et al. 2019; McDonald and Pizzari 2018). Spatial avoidance of males via perches or hiding places is one way to greatly reduce the number of sexual interactions that take place, though this may be costly to females if it prevents them carrying out normal behaviours (McDonald et al. 2019). Additionally, females may solicit copulations, or they may accept or resist copulation attempts from males.

Females might change strategies to retain some control of copulation according to different socio-sexual scenarios. For instance, if males are highly motivated to mate, and sexual harassment is high, it may be worthwhile remaining perched above the ground for long periods of time to prevent forced copulations. If male sexual motivation is lower, it may be possible to retain adequate control of copulations using less extreme methods, such as differential solicitation of desired males.

Female dominance and age are likely to have complex, interacting effects on mating strategies and the influence of novelty. Age and dominance status are strongly correlated in female junglefowl, with older females usually occupying the higher positions of hierarchies (Kim and Zuk 2000; McDonald et al. 2019), which means the effects of each may be difficult to discern separately.

Younger, less dominant females are more polyandrous, receiving more sexual attention from males (McDonald et al. 2019). This is likely to affect female strategies. For instance, as discussed in Chapter 1, if a female receives more attempts, then more resistance is needed in order to achieve the same number of copulations. Different levels of resistance seen may therefore reflect different routes to achieving the same optimal number of copulations or level of polyandry.

Aims of this chapter

In this chapter, I use an experimental approach to investigate female responses to male novelty in replicate red junglefowl flocks. As a secondary aim, I test for potential roles of female age and social status in modulating such responses.

The effect that we would expect to see depends on the relative weights of costs of copulation and benefits of polyandry in this species. We would expect a Coolidge-type effect (receptivity decreasing over time and then increasing when presented with novel partners) if the cumulative benefit to females increased with each partner, while costs scaled per copulation largely indifferent to the number of partners.

By contrast, if there is a strong increase in costs with each new partner, then we would instead expect to see a preference for familiar mates and a resistance to switching – akin to an 'anti-Coolidge' effect. If polyandry is fitness-neutral, then we would expect to see no change to receptivity when new males are introduced. We would expect the previous pattern (perhaps a decrease in receptivity in response to copulation costs, or perhaps a steady level of receptivity over time) to continue unaffected.

Since these costs and benefits are likely to be affected by age and social status, we expect that responses to partner novelty may also vary according to these factors.

Methods

Experimental setup

A population of captive red junglefowl (*Gallus gallus*) at the John Krebs Field Station near Oxford, UK was observed during the breeding seasons (early April to late August) of 2019 and 2021. Five experimental repeats were carried out each year. Each experimental repeat took place over nine days and involved behavioural observations over two hours each morning and evening from 8.00-10.00 and from 16.30-18.30. Sexual activity in this species shows a large peak in the evening and a secondary peak early in the morning (Løvlie and Pizzari 2007); these observation times were the earliest and latest that were practical with regard to available transport.

For each experimental repeat, six female junglefowl were released into an enclosed outdoor pen at the start of the afternoon observation session on day 1. This pen is mostly open space, with branches and pallets propped against walls to allow perching and hiding, and a natural soil floor covered with gravel and bark to allow foraging and dust bathing. Birds had continuous access to feed (Small Holder Range Layers Pellets) and water, as well as foraged plant material and small invertebrates.

Table 1: *Schedule of experimental observation sessions. Yellow = female only sessions; dark blue = first group of males; light blue = second group of males.*

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
AM		2	4	6	8	10	12	14	16
PM	1	3	5	7	9	11	13	15	

The six females were allowed to acclimatise for two days, during which aggressive interactions were recorded during observation sessions 1-4 to determine individuals' dominance status. A group of three males was added to the pen at the start of observation session 5, and sexual interactions were recorded during sessions 5-10. This first set of males was removed from the pen at the end of observation session 10, and later the same day, a new set of males was added to the pen at the start of session 11. Sexual interactions were again recorded over the next three days until the end of the experiment. ***Table 1*** illustrates the schedule of observation sessions. Prior to the experiment, all birds had been sexually rested for a minimum of three days, usually longer.

The ratio of females to males was chosen based on advice from T. Pizzari, and was intended to balance the welfare of females (too many males per female would mean high levels of harassment) and gathering sufficient data (too few males per female would mean low levels of mating interaction).

Social status

Aggressive interactions between females were recorded during the female-only sessions (observation sessions 1 – 4), and aggressive interactions between males were recorded during the sessions in which males were present (sessions 5 - 16). Behaviours recorded are defined in ***Table 2***.

Table 2: *Aggressive behaviours of females and males that were recorded to determine dominance status.*

Behaviour (female)	**Description**
Displace	Aggressor moves towards stationary target to within roughly one metre. Target moves away by at least one body length within ten seconds. No physical contact occurs between the birds.
Peck	Rapid downward motion of aggressor's beak directed towards target. Do not include exploratory or grooming pecks, which are gentler (usually closer range, may be upward direction).
Lunge	Jabbing motion of entire body of aggressor towards target. Aggressor may run at target but does not chase after her.
Chase	The aggressor runs after the target with both running simultaneously in the same direction for at least three paces.
Behaviour (male)	**Description**
Chase, peck, run or jump at another male	As described for females.
Waltz	Aggressor walks in an arc in front of target with one wing outstretched, and beating this wing on his tarsus.

Interrupt	Aggressor physically approaches target, to within touching distance, when target is copulating or attempting to copulate. Aggressor may attack target or insert himself between the copulating pair.
Fight	A pair of males jump and peck at each other. Between bouts they face each other with heads lowered and neck feathers raised. The winner is the last to stop and walk away.

Elo scores were calculated using the package *EloRating* in R (Neumann and Kulik 2020), with a starting score of 1000 for each bird, a maximum gain or loss of 100 per interaction, and equal weighting for each interaction type. Raw Elo scores ranged from 323 to 1725 for females (median 1028) and 535 to 1520 for males (median 930.5). Rescaled Elo scores (range: 0 - 12) were used for subsequent analysis.

Age

All birds used in this experiment fell into one of three age categories:

- Young: one year old
- Middle-aged: three or four years old
- Old: five or six years old.

The groups of six females used in this experiment were age structured, with two “old” females and either four “middle-aged” females (2019 replicates) or four “young” females (2021 replicates).

Old females had significantly higher adjusted Elo scores than both young and middle-aged females. Differences between young and middle-aged females (who were never used together) were not significant. (ANOVA: F = 21.82, $p < 0.0001$, followed by Tukey Honest Significant Differences test for pairwise comparisons, adjusted p values: Old-Young $p < 0.0001$, Old-Mid $p < 0.0001$, Young-Mid $p = 0.988$).

Male groups were not age-structured as part of the experiment. All males used were either “old” (2021 replicates) or a mixture of “middle-aged” and “old” (2019 replicates). Previous research has shown a lack of relationship between male age and dominance in female-biased groups of feral fowl (Dean et al. 2010).

These discrepancies in age structure were dictated by limitations in the availability of birds of different age cohorts in the study population.

Measuring female propensity to mate

Female chickens and junglefowl solicit copulation by crouching in a stereotyped posture that facilitates mounting by males (interaction type 1- see ***Table 3*** and ***Figure 1***). However, only a small proportion of sexual interactions (below 5% in this study) are typically initiated by females in this way (Løvlie and

Pizzari 2007). The remainder of copulation attempts are initiated by males. Male-initiated attempts may be forced, where males chase and/or grab females, or un-forced, where males approach a female in a stereotyped manner which she can easily avoid (see ***Table 3***).

Figure 1: *A female red junglefowl in the experimental pen adopts the crouched soliciting posture. Photograph: Lorena Boisseau.*

In this study, we characterise all sexual interactions as one of the three types detailed in ***Table 3***. This gives us three different measures of female receptivity:

1. Number of solicitations made by females (count).

2. Acceptance rate of un-forced attempts (binary variable: "accept" if she crouches to facilitate copulation, "resist" if she does not).

3. Acceptance rate of forced attempts (binary variable: "resist" if she resists as described in ***Table 3***, "accept" if she passively allows copulation).

Table 3: *Types of sexual interaction in red junglefowl, and the level of female control over each.*

Interaction type	Description	Level of female control
1. Females solicit copulation by crouching	Females solicit copulations by assuming a stereotyped crouched posture near to males (see ***Figure 1***). This behaviour is relatively rare, but almost always results in successful copulation.	**High.** This behaviour is under full female control. Females can directly choose mating partners.
2. Males approach females (non-forced)	Males initiate copulation by approaching females from behind with their hackle feathers raised. This most commonly occurs when a female is foraging in close physical proximity to a male. Females can accept copulation by crouching, or terminate the copulation attempt by stepping away.	**Intermediate.** Females can easily prevent copulation by stepping away; in addition, this type of interaction occurs primarily when females choose to socially associate with males. However, it is not under direct female control as the first type of interaction is.
3. Males attempt to force copulation	Males attempt copulation by chasing, lunging at, grabbing or trying to grab females and mounting without a copulation having been invited by crouching. Females are targeted for forced copulation attempts whether or not they are in close physical proximity to males. Resisting this type of attempt can involve running away, struggling to free herself, or uttering distress calls, which often attract intervention from dominant males if the attempt is made by a subordinate male (Løvlie, Zidar, and Berneheim 2014).	**Low.** Females can resist forced copulations, but may not be successful in preventing them. They can reduce the number of forced copulation attempts by hiding or perching, but this may be costly and is non-selective (resists all males).

Copulations were determined to be successful if cloacal contact was either observed or inferred based on tail position. Attempts which did not involve cloacal contact were determined to be unsuccessful.

Time spent on perches

This species is informally reported to spend time on perches as a means of escape from aggression, including sexual harassment. McDonald et al. (2019) found that older red junglefowl females spent more time perching than younger females, and associated less with males and received less sexual harassment. When perched above the ground, females are subject to fewer copulation attempts by males and it is difficult for males to force copulation. Spending time on perches may therefore represent an effective form of resistance. However, this may be costly for the female as activities such as foraging, dust bathing, and nesting are prevented.

During observations in 2021 (5 trials), instantaneous scan samples were taken every five minutes, and the identity of any females on perches noted. There was abundant space to perch in several locations around the experimental pen, so that it was possible for all females to perch at once. The number of times a female was seen perching (with a possible maximum of 24 per session) is used as an indicator of her motivation to avoid copulation attempts. Combining this measure with the three direct measures of copulation propensity gives a fuller picture of the methods used by females to influence copulation rate.

Statistical analysis

Firstly, to put female behaviour in context, we examined influences on the number of male copulation attempts of both types (forced and non-forced). Secondly, we examined sources of variation in the female acceptance rate of forced and non-forced attempts, and in the number of times females solicited copulation by crouching. Thirdly, we examined sources of variation in the number of times a female was seen perching.

Generalised linear mixed effects models were fit for each of these outcome variables using the package *lme4* (Bates et al. 2015). See ***Table 4*** for details of each model's specification. For each variable, a Poisson data structure was used, unless the data were significantly overdispersed (variance around 1.5 times or more greater than the mean) or zero-inflated (zero values over 2% more frequent than expected), in which case a negative binomial model was fit using the package *glmmTMB* (Zeileis, Kleiber, and Jackman 2008).

Dispersion formulae and zero inflation formulae were trialled in these models to account for unevenness across variables, but for all models except models of perching these were not found to be necessary: model fit was acceptable without them, and inclusion of these additional specifications did not significantly improve AICc scores. For models of perching, a zero-inflation term was specified to account for high, variable levels of zero-inflation. All fixed effects were included in the zero-inflation terms. (Burnham, Anderson and Huyvaert, 2011; Symonds and Moussalli, 2011; Brooks *et al.*, 2017; Blasco-Moreno *et al.*, 2019).

Each outcome variable is modelled over successive observation sessions in the experiment, so session number was encoded as a fixed effect. In null models for female behaviour, the assumption is that female behaviour is not affected by male novelty; ie that the replacement of the first group of males with the second group is irrelevant with respect to dependent variables. Male group (group 1 or group 2) is therefore not included in null models (see ***Figure 2*** and ***Table 4*** for further explanation).

Each model contained fixed effects for whether the session was morning *versus* evening (since behaviour is expected to vary throughout the day (Løvlie and Pizzari 2007)), female age (old > mid > young), and female and male adjusted Elo scores. For female responses to male copulation attempts, the number of attempts received was used as an offset variable, so that models of number of acceptance events became models of acceptance rates. The number of attempts was also used as an additional fixed effect, allowing female acceptance rates to vary according the number of attempts they received.

Female solicitations were modelled in two ways to ensure robustness of conclusions. Firstly, the number of solicitations was modelled using the negative binomial distribution. Secondly, due to the low occurrence of solicitations, a high proportion of values in this dataset were zero, so we additionally modelled the probability of a female soliciting, according to a logistic distribution of zero *versus* non-zero values.

A multi-stage approach was taken with modelling female perching. The number of times a female was seen perching per session was modelled in the same way as other variables for the subset of sessions where males were present. Additionally, the number of scans in which females were observed perching was modelled for all sessions including those where males were not present, with male presence/absence rather than group number included as a predictor.

For all models, the maximum supported random effect structure was included, starting with a random intercept for replicate number, and then a random intercept for female ID followed by male ID (opposite order for male behaviours). In the experiment, individual birds were sometimes re-used in different replicates. The random effect was specified such that an individual bird is treated as the same individual if used in two different replicates in the same field season, but as two different individuals if re-used in the 2021 field season after having been previously used in 2019.

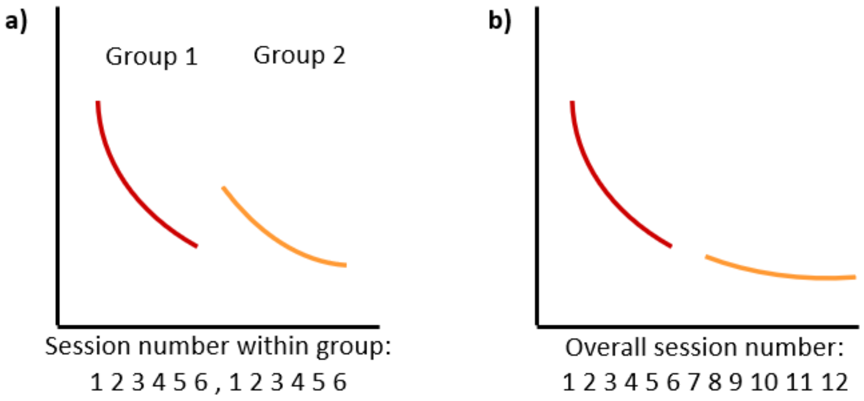
All models were checked for outliers, dispersion, zero-inflation, and evenness of residuals using simulation-based tests in the package *DHARMa* (Hartig 2020). Goodness of fit was tested with the Kolgorov-Smirnov test (Berger and Zhou 2014). To establish the significance of predictor variables, likelihood ratio tests were then run between full models and reduced models with a specific predictor missing (Lewis, Butler, and Gilbert 2011).

For each outcome variable used as an indicator of female receptivity, the first step was to determine whether there was a change over time after first exposure to males. The *a priori* assumption, based on knowledge of the Coolidge effect in males, was that any change over time would most likely be represented by an exponential decay, rather than a linear change (Pizzari et al. 2003). We tested whether adding a term for exponential decay over successive observation sessions significantly improved model fit, compared to models where outcomes were assumed to be consistent across observation sessions.

Next, we tested for any effects dependent on male sexual novelty. If novelty significantly affects behavioural outcomes, we would expect those outcomes to be better explained by a model that took into account the replacement of familiar males with novel ones. We therefore tested whether a model that allowed different slopes and intercepts for group 1 and group 2 provided a better fit than a model which simply assumes a change over time, in effect constraining groups 1 and 2 to fit into a single slope (***Figure 2***). If there is no significant novelty effect, then we expect the two models to be equivalent.

Note that this does not necessarily imply a Coolidge-like effect, merely that there is some change to the trajectory when familiar males are replaced by novel ones. An anti-Coolidge effect, where females prefer familiar males and resist novel ones, would also be better described by two trajectories than one. This procedure therefore tests for *any novelty effect*: determining the direction of the effect is done by visual inspection of the data.

Figure 2: *Hypothetical trajectories of some measure of female copulation propensity declining over time across the first and second groups of males with (a) and without (b) a novelty effect. The red line represents the response to the first group, and the orange line the response to the second group. The labels on the graphs (group, session within group, overall session) explain how session number is encoded in the null (b) and alternative (a) models. If there is no effect of novelty (as in graph b), then modelling female response to the first and second groups of males will be equivalent to modelling female response simply as a function of time since first exposure to males (model b).*



MODEL A: Response variable ~ log(session within group) + group + log(session within group) * group

MODEL B: Response variable ~ log(overall session)

The number of attempts by males was studied largely as background for interpreting female receptivity. It was assumed that male behaviour patterns would be similar for the first and second groups of males, implying that these should be modelled as a function of session within group, rather than overall session (***Figure 2***). These models were tested to determine whether there were significant differences between the patterns shown by the first and second groups of males.

The number of successful copulations was treated in the same way. We inferred that any differences in patterns of copulation frequency between the first and second groups of males may reflect female influence over copulation frequency, because we assumed that male propensity to copulate did not vary systematically between the first and the second male group.

Full specifications for null and alternative models for all outcome variables are given in ***Table 4***.

Table 4: *Model specifications used for main analyses. See main text for details of the logical process of model fitting. "Session" = overall session within the experiment, ranging from 1-12 across groups 1 and 2 together, "Group.sesh" = session within the group, ranging from 1-6 for group 1 and group 2 (see* ***Figure 2****).*

Male behaviours & outcomes		
Variable modelled	Model without difference between groups	Model with difference between groups
Number of un-forced attempts	`Approaches ~ log(Group.sesh) + Time + Elo.m + Elo.f + Age.f + (1|Replicate) + (1|Year:Male.ID) + (1|Year:Female.ID), family = nbinom1()`	`Approaches ~ log(Group.sesh) + Group + log(Group.sesh) * Group + Time + Elo.m + Elo.f + Age.f +(1|Replicate) + (1|Year:Male.ID) + (1|Year:Female.ID), family = nbinom1()`
Number of forced attempts	`Grabs~ log(Group.sesh) + Time + Elo.m + Elo.f + Age.f + (1|Replicate) + (1|Year:Male.ID) + (1|Year:Female.ID), family = nbinom1()`	`Grabs ~ log(Group.sesh) + Group + log(Group.sesh)*Group + Time + Elo.m + Elo.f + Age.f + (1|Replicate) + (1|Year:Male.ID) + (1|Year:Female.ID), family = nbinom1()`
Successful copulations	`All.succ ~ log(Group.sesh) + Time + Elo.f + Elo.m + Age.f +(1|Replicate) + (1|Year:Female.ID) + (1|Year:Male.ID),`	`All.succ ~ log(Group.sesh) + Group + Group * log(Group.sesh) + Time + Elo.f + Elo.m + Age.f +`

<table>
<tr><td></td><td>family = nbinom1()</td><td>(1|Replicate) + (1|Year:Female.ID) + (1|Year:Male.ID), family = nbinom1()</td></tr>
<tr><td colspan="3">Female sexual behaviours</td></tr>
<tr><td>Variable modelled</td><td>Model without novelty effect</td><td>Model with novelty effect</td></tr>
<tr><td>Number of solicitations</td><td>Crouches ~ log(Session) + Time + Elo.f + Elo.m + Age.f + (1|Replicate) + (1|Year:Female.ID), family = nbinom1()</td><td>Crouches ~ log(Group.sesh) + Group + log(Group.sesh)*Group + Time + Elo.f + Elo.m + Age.f + (1|Replicate) + (1|Year:Female.ID), family = nbinom1()</td></tr>
<tr><td>Number of solicitations (logistic model)</td><td>Crouchprob ~ log(Session) + Time + Elo.f + Elo.m + Age.f +(1|Replicate) + (1|Year:Female.ID), family = binomial(link = "logit")</td><td>Crouchprob ~ log(Group.sesh) + Group + log(Group.sesh)*Group + Time + Elo.f + Elo.m + Age.f +(1|Replicate) + (1|Year:Female.ID), family = binomial(link = "logit")</td></tr>
<tr><td>Acceptance rate of un-forced attempts</td><td>Accept ~ offset(log(Approaches)) + log(Session) + Approaches + Time + Elo.f + Elo.m + Age.f +(1|Replicate), family=poisson()</td><td>Accept ~ offset(log(Approaches)) + log(Group.sesh) + Group + log(Group.sesh)*Group + Approaches + Time + Elo.f + Elo.m + Age.f + (1|Replicate), family=poisson()</td></tr>
<tr><td>Acceptance rate of forced attempts</td><td>Forced.accepts ~ offset(log(Grabs)) + log(Session) + Grabs + Time + Age.f + Elo.f + Elo.m + (1|Replicate) + (1|Year:Female.ID), family = poisson()</td><td>Forced.accepts ~ offset(log(Grabs)) + log(Group.sesh) + Group + Group*log(Group.sesh) + Grabs + Time + Age.f + Elo.f + Elo.m + (1|Replicate) + (1|Year:Female.ID), family = poisson()</td></tr>
<tr><td colspan="3">Female perching</td></tr>
<tr><td rowspan="2">Perching (only sessions with males present)</td><td>Without novelty effect</td><td>With novelty effect</td></tr>
<tr><td>Perches ~ log(Session) + Time + Age.f + Elo.f</td><td>Perches ~ log(Group.sesh) + Group + Group * log(Group.sesh) +</td></tr>
</table>

	`+ (1|Replicate)`
	`+ (1|Female.ID),`
	`zi = ~ log(Session) + Time + Age.f + Elo.f,`
	`family = nbinom1()`
		`Time + Age.f + Elo.f`
		`+ (1|Replicate)`
		`+ (1|Female.ID),`
		`zi = ~ log(Group.sesh) + Group + Group * log(Group.sesh) +`
		`Time + Age.f + Elo.f,`
		`family = nbinom1()`

Perching (all sessions including those prior to addition of males)	Without effect of males	With effect of males

	`Perches ~`
	`Time + Age.f + Elo.f + (1|Replicate)`
	`+ (1|Female.ID),`
	`zi = ~ Time + Age.f + Elo.f,`
	`family = nbinom1()`
		`Perches ~`
		`Males + Time + Males * Time + Age.f + Elo.f`
		`+ (1|Replicate)`
		`+ (1|Female.ID),`
		`zi = ~ Males + Time + Males * Time + Age.f + Elo.f,`
		`family = nbinom1()`

Results

Male behaviour

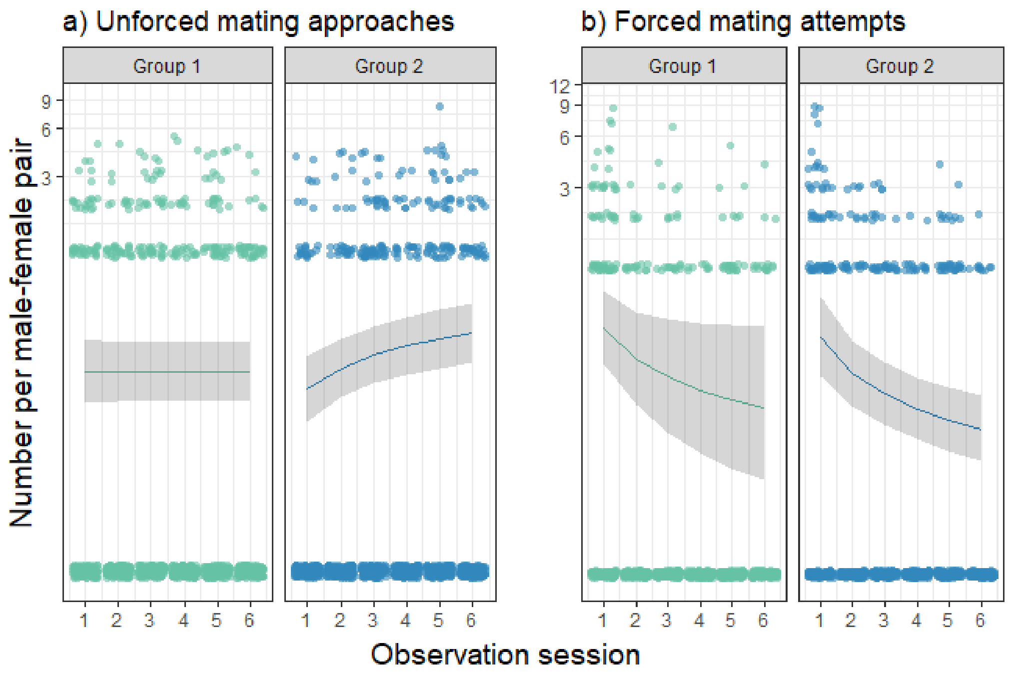
The number of male copulation attempts, both un-forced approaches (henceforth "approaches") and attempts at forced copulation (henceforth "forced copulations"), varied across sessions within each group of males (**Approaches:** χ^2= 19.647, df = 2, p < 0.0001, **Forced copulations:** χ^2= 96.632, df = 2, p < 0.001; likelihood ratio test between full models as detailed in ***Table 4*** and reduced models with "session within group" not included as a predictor variable). For approaches, the pattern differed significantly between the first and second groups of males, with a slight increase over successive sessions in the second, but not the first, group (χ^2= 13.327, df = 2, p = 0.001; likelihood ratio test between full model where slope and intercept differ by group and reduced model where slope and intercept are constrained to be the same for both groups- see ***Table 4***).

For forced copulations, the pattern in both groups resembled a decline over time. This pattern did not significantly vary the between first and second groups of males (χ^2 = 1.537, df = 2, p = 0.464; likelihood ratio test as described above, see ***Table 4***).

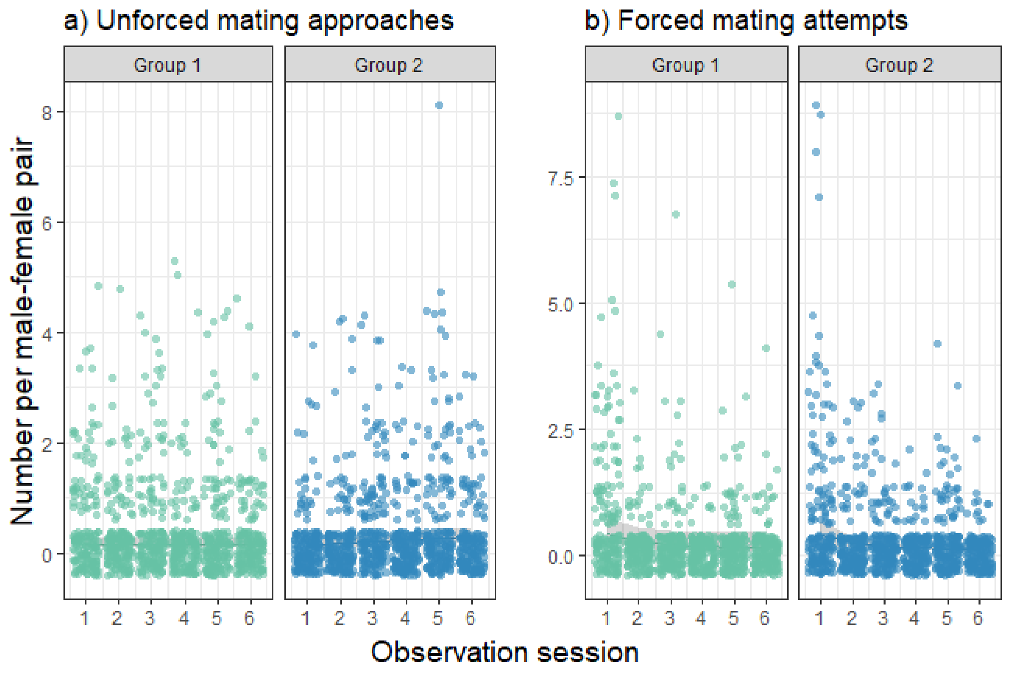
Both types of attempt by males were significantly more likely to be observed in evening than morning sessions (**Approaches:** χ^2= 39.270, df = 1, p < 0.001, **Forced copulations:** χ^2= 53.450, df = 1, p < 0.001, ***Figure 3***).

***Figure 3:** Variation in frequency of both types of male copulation attempt across observation sessions. Points represent the number of attempts made per male-female pair in a given observation session (raw data); lines and confidence bands are model predictions (models specified in second column of **Table 4**). **4a)** Unforced approaches; **4b)** attempts at forced copulation. These results are plotted first on a log scale **(i)** and then on a linear scale **(ii)**: the former allows model predictions to be more clearly seen, and the latter makes the true scale of observations and predictions clearer.*

i)



ii)

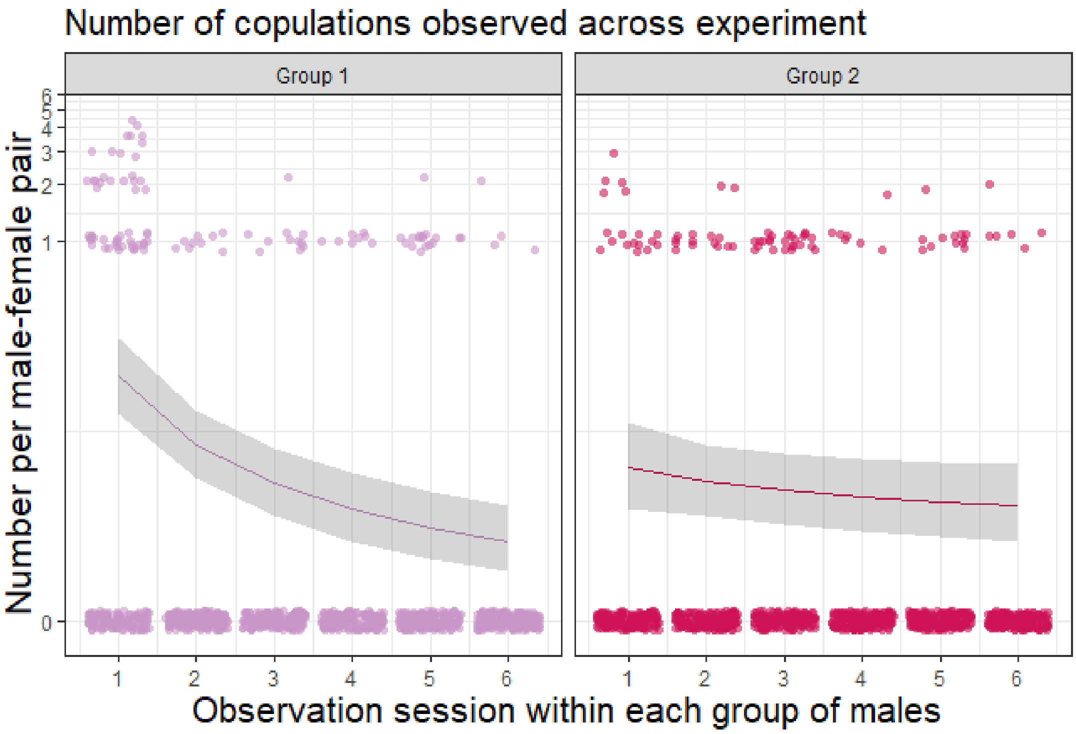


Copulation rate

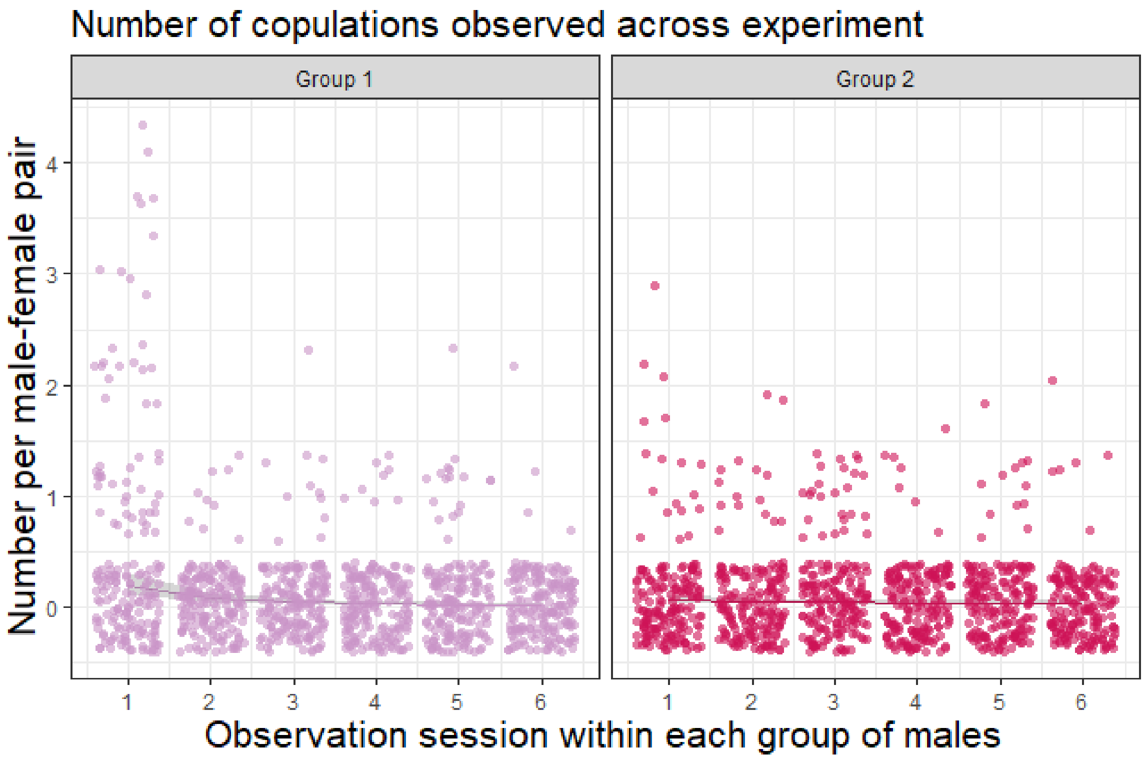
The number of successful copulations declined significantly after groups of males were first introduced (χ^2 = 68.850, df = 2, p < 0.001). However, different patterns were observed between groups 1 and 2 (see ***Figure 4***). The second group of males had a lower number of copulations (χ^2 = 3.924, df = 1, p = 0.048), and a shallower decline over time (χ^2 = 19.004, df = 1, p < 0.001). In the sessions where males are first added, for the first group there was a mean of 0.511 copulations per male-female pair, dropping to just 0.133 for the second group (d = 0.496).

A significant difference was seen in the copulation rate in morning and evening observation sessions, with a mean of 0.050 successful copulations per male-female pair in morning sessions and 0.170 in evening sessions (d = 0.296; likelihood ratio test comparing models with and without time as a predictor gives χ^2 = 22.109, df = 1, p < 0.001).

Figure 4: *Change in copulation number of copulations seen per male-female pair over successive sessions. Points are raw numbers, line and confidence band are model predictions of the full model (second column in **Table 4**). The pattern of copulation frequency across successive sessions showed different patterns for the first and second group of males (see main text). These results are plotted first on a log scale **(i)** and then on a linear scale **(ii)**: the former allows model predictions to be more clearly seen, and the latter makes the true scale of observations and predictions clearer.*



ii)



Female copulation propensity

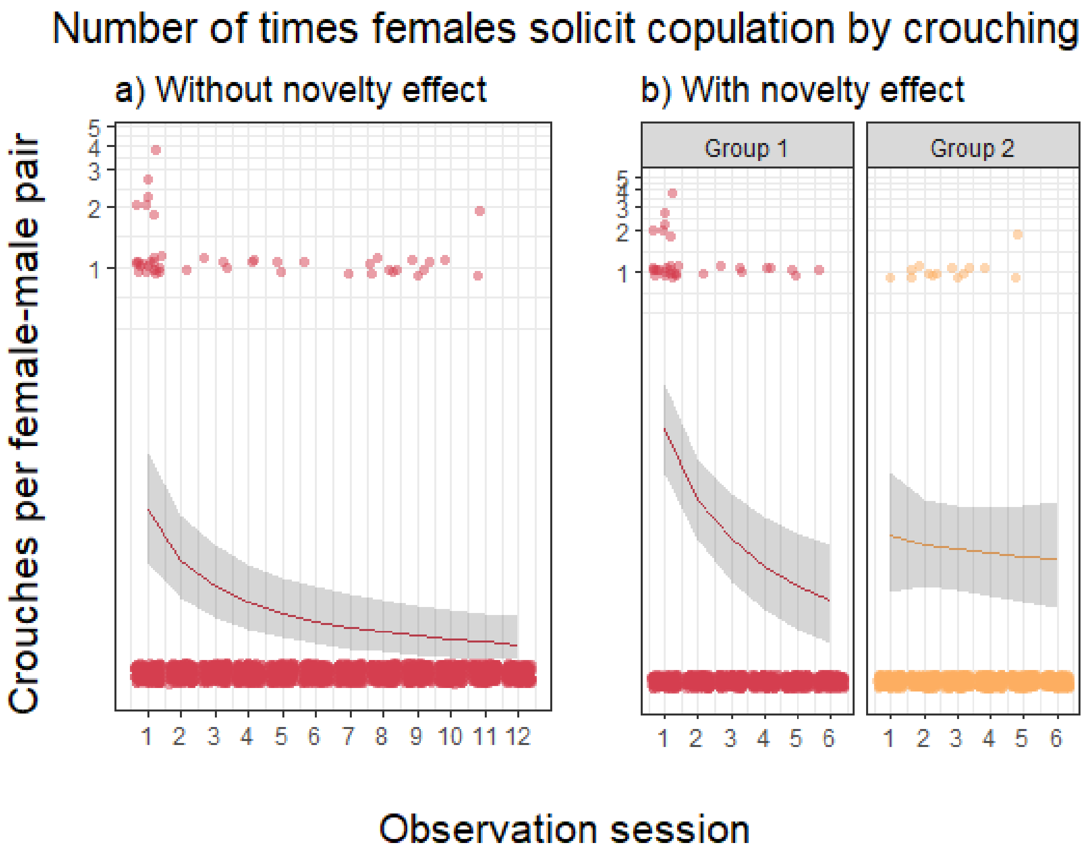
Female propensity to copulate declined significantly with time since exposure to males in all measures studied (**Crouches:** χ^2 = 35.885, df = 1, p < 0.001 **Approaches:** χ^2 = 33.455, df = 1, p < 0.001 **Forced copulations:** χ^2 = 7.2777, df = 1, p = 0.007; test statistics obtained by likelihood ratio test between models that included log of overall session number as a fixed effect (first column in ***Table 4***) and models with this term removed).

In all measures of copulation propensity, logarithmic decay provided a better fit than linear decay (**Crouches:** ΔAICc = 11.655 **Approaches:** ΔAICc = 9.382 **Forced copulations:** ΔAICc = 1.577).

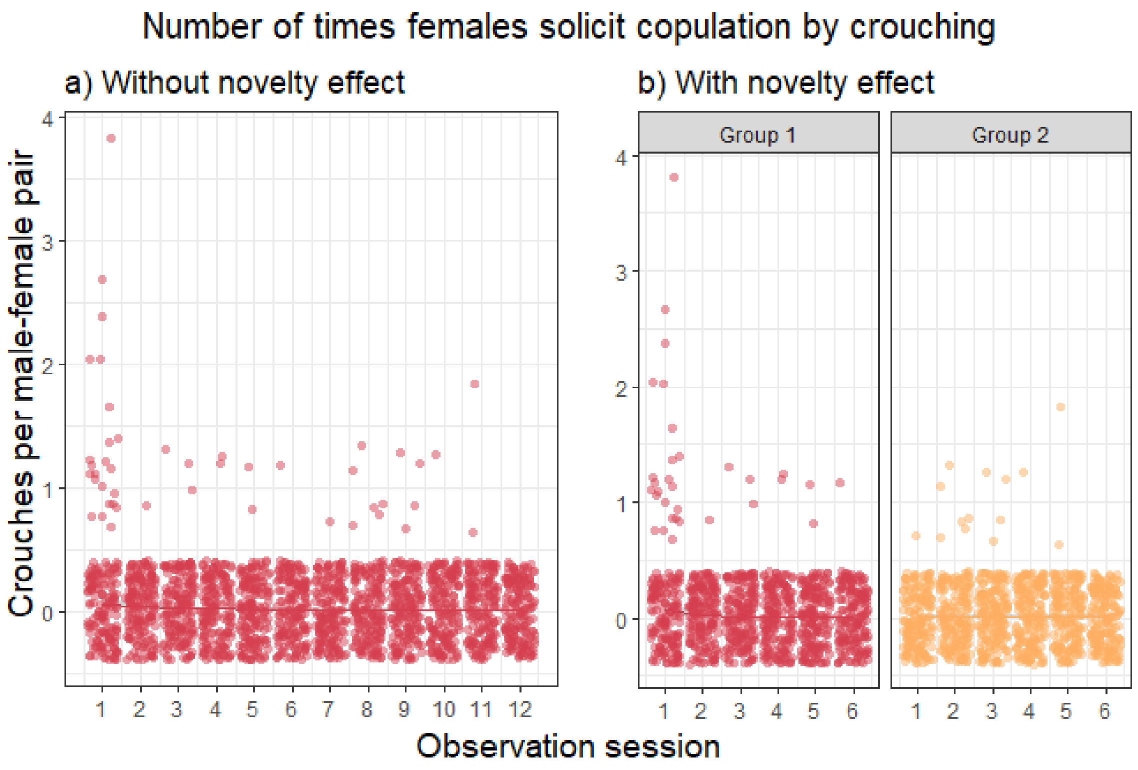
Time of day did not significantly affect any of these behaviours: females were equally likely to solicit copulation or to accept male copulation attempts in the morning as in the evening (**Crouches:** χ^2 = 1.795, df = 1, p = 0.180 **Approaches:** χ^2 = 2.285, df = 1, p = 0.131 **Forced copulations:** χ^2 = 0.005, df = 1, p = 0.945; test statistics obtained by likelihood ratio test between full model (second column in ***Table 4***) and model with Time variable removed).

Figure 5: *Number of times females solicit copulation by crouching over the course of the experiment. Each data point is the number of crouches per female-male pair in a given observation session. Plotted lines and confidence bands are the predictions given by different explanatory models.* ***5a)*** *is the model that fits all data points to a single curve over successive sessions (i.e. no potential for novelty effect).* ***5b)*** *allows different intercepts and slopes for the first and second groups of males (i.e. has the potential to show a novelty effect). Since the likelihoods of the two models are significantly different ($\chi^2 = 7.4558$, df = 2, p = 0.024), we can say that there is evidence for a novelty effect in this measure of female copulation propensity. These competing models are plotted first on a log scale* ***(i)*** *and then on a linear scale* ***(ii)****: the former allows model predictions to be more clearly seen, and the latter makes the true scale of observations and predictions clearer.*

i)



ii)

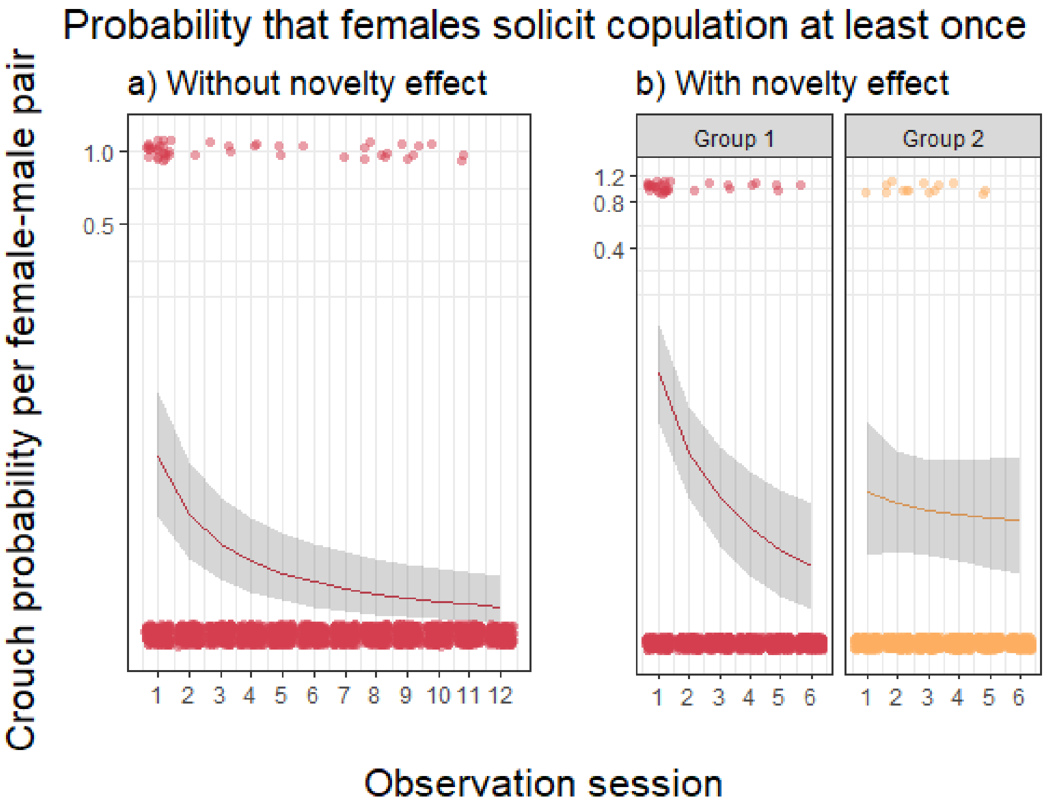


There was evidence for an effect of male novelty on female propensity to solicit copulations (***Figure 5***). A significant difference was detected between models A and B (see ***Figure 2*** and ***Table 4***) when modelling this variable ($\chi^2 = 7.4558$, df = 2, p = 0.024). We can compare the predictions of these two models in session 7, the session where the first group of males are replaced by a second group. The model that allows for a novelty effect (ie the model that contains information about two separate groups) predicts 0.014 solicitations per male-female pair in this session (CI: 0.005 – 0.042). On the other hand, the model that does not allow for a novelty effect and assumes female responses follow a single trajectory across the experiment predicts 0.010 solicitations per pair in this session (CI: 0.005 – 0.022).

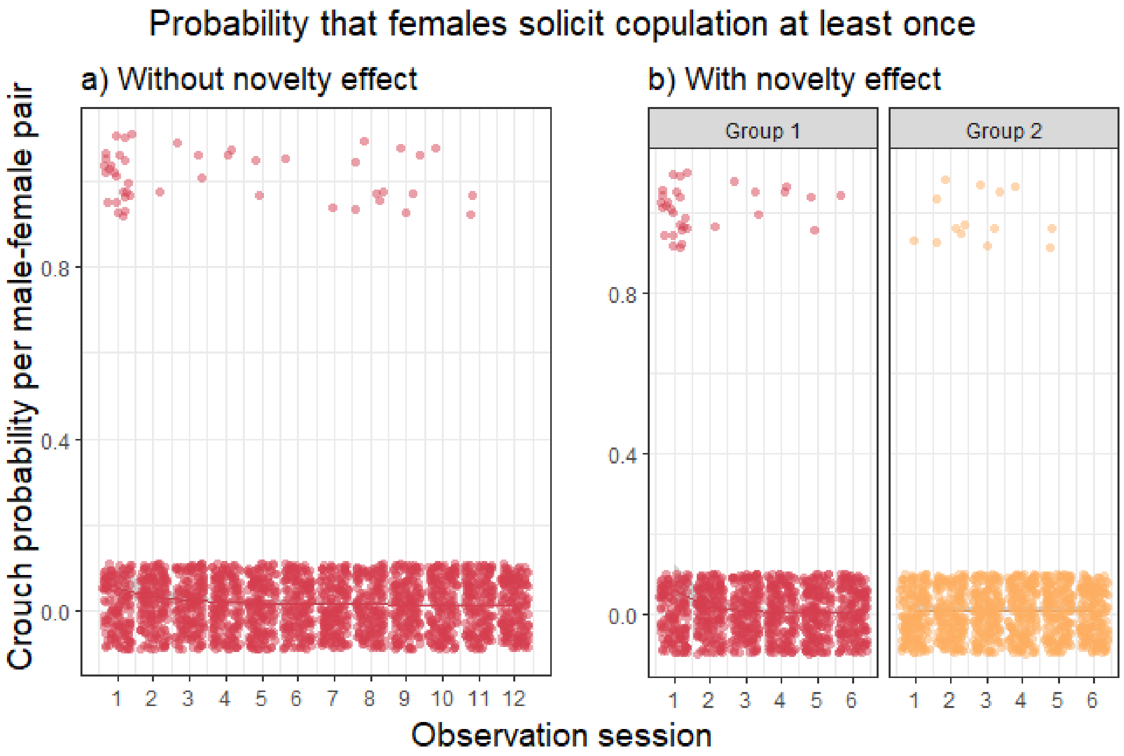
A significant novelty effect was also found for **Crouches** when modelled with logistic regression as a binary probability of crouching at least once versus not at all ($\chi^2 = 7.594$, df = 2, p = 0.022). Here, the predicted probability of crouching at least once in any female-male pair in session 7 (the session when the second group of males is introduced) was 0.008 when not accounting for potential novelty effects (CI: 0.004 – 0.019), and 0.012 when the novelty of the second group of males is accounted for (CI: 0.004 – 0.035). See ***Figure 6***.

Figure 6: *Raw data (points) and model predictions (lines and confidence bands) for logistic models of female solicitation of copulations, without (**6a**) and with (**6b**) novelty effects (see caption of **Figure 5** for explanation). Y axes represent probability that at least one solicitation occurred per female-male pair per observation session. Model likelihoods differ significantly ($\chi^2 = 7.594$, $df = 2$, $p = 0.022$), suggesting that a novelty effect is seen in this measure of female copulation propensity. These competing models are plotted first on a log scale **(i)** and then on a linear scale **(ii)**: the former allows the model predictions to be more clearly seen, and the latter makes the true scale of observations and predictions clearer.*

i)

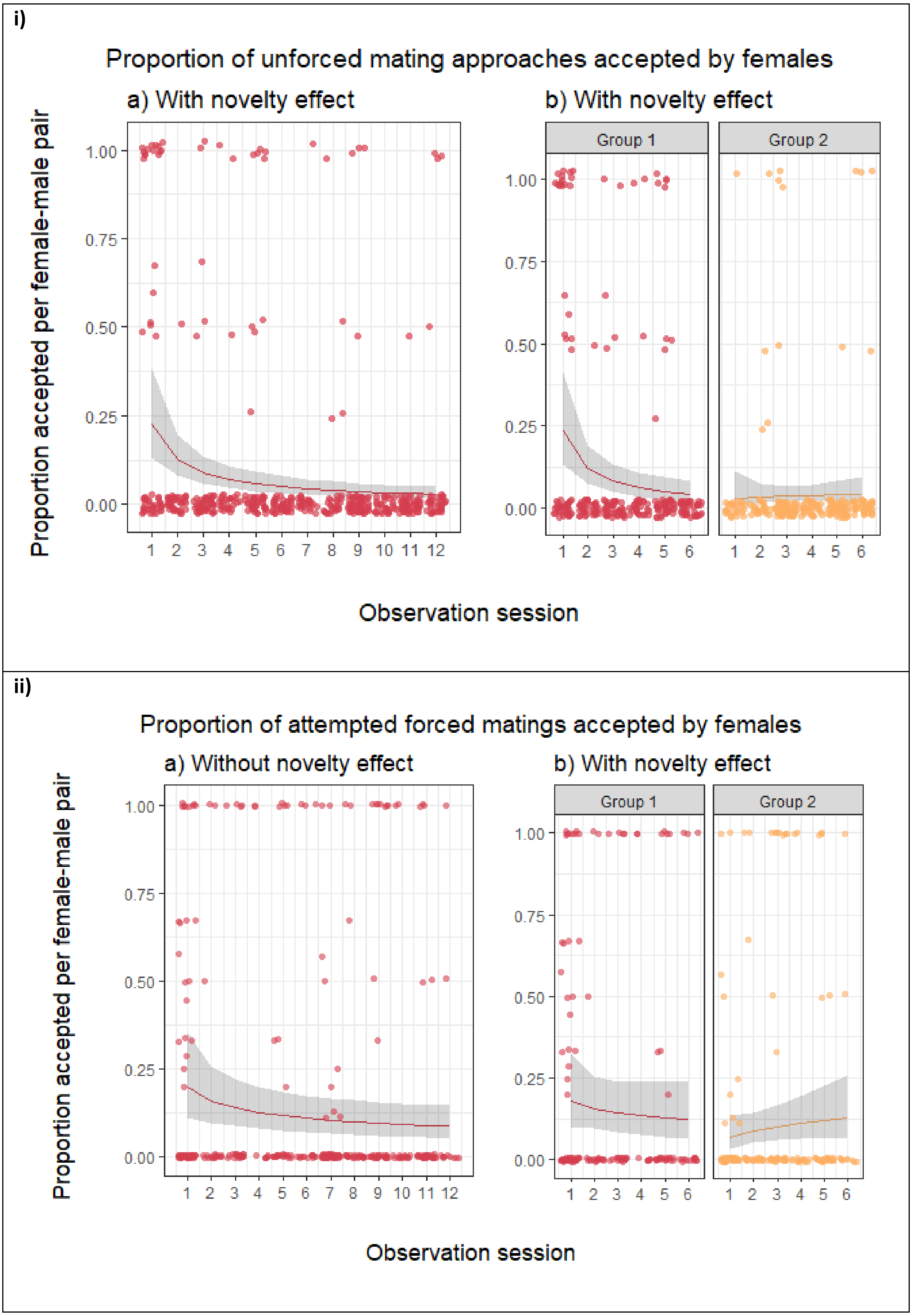


ii)



The other two measures of female copulation propensity did not show any significant difference between models A and B as outlined in ***Figure 2*** and ***Table 4*** (**Approaches:** χ^2 = 2.599, df = 2, p = 0.273; **Forced copulations:** χ^2 = 2.950, df = 2, p = 0.229). This implies that models assuming a simple decline over time, with no effect of novelty, were sufficient to explain the data, i.e. that there was no significant novelty effect. See ***Figure 7***

Figure 7: *Proportion of copulation attempts by males that females accept over the course of the experiment (Upper plots show unforced copulation attempts, lower plots show attempts at forced copulation). Each data point represents the proportion for one female-male pair in a given observation session where at least one attempt of that type took place.* ***a)*** *show model predictions assuming no novelty effect.* ***b)*** *show predictions that allow a novelty effect. Given the likelihoods of these models are not significantly different (**Approaches:** χ^2 = 2.599, df = 2, p = 0.273; **Forced copulations:** χ^2 = 2.950, df = 2, p = 0.229), these results do not support a novelty effect in either of these two measures of female copulation propensity.*


i)
Proportion of unforced mating approaches accepted by females
a) With novelty effect
b) With novelty effect
Group 1
Group 2
Proportion accepted per female-male pair
1.00
0.75
0.50
0.25
0.00
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5 6
Observation session
ii)
Proportion of attempted forced matings accepted by females
a) Without novelty effect
b) With novelty effect
Group 1
Group 2
Proportion accepted per female-male pair
1.00
0.75
0.50
0.25
0.00
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5 6
Observation session

Perching

Perching frequency was higher when males were present (χ^2 = 49.883, df = 2, p < 0.001; likelihood ratio test between model with no effect of males (first column in ***Table 4***) and alternative model including fixed effect for male presence or absence). Females were seen perching an average of 3.189 times per session when males were present, and 1.125 times per session when males were absent (d = 0.245).

When males were present, females perched significantly more often in the evening (χ^2 = 25.585, df = 2, p < 0.001). There was a mean of 4.556 times observed perching per female in the evening compared to just 1.822 in the morning (d = 0.546). However, the direction of this difference is flipped when males are not present: in these sessions, we see a mean of 2.013 times observed perching per female in morning sessions and only 0.167 in evening sessions (d = 0.651). Confirming this pattern, the model including all sessions revealed a significant interaction between time of day and presence of males (χ^2 = 33.311, df = 2, p < 0.001; comparing full model (second column in ***Table 4***) with reduced model with no interaction term). See ***Figure 8*** for illustration.

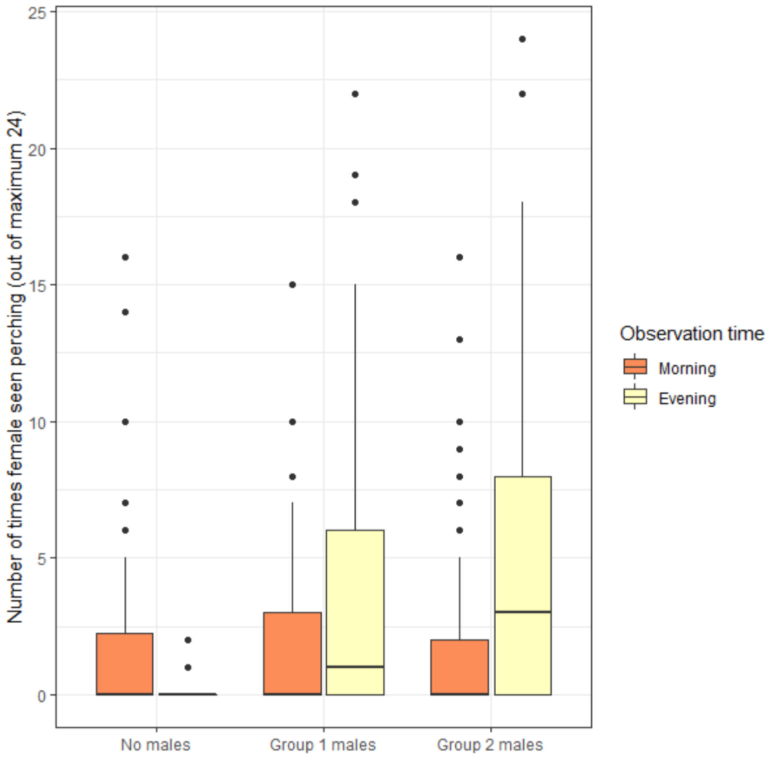
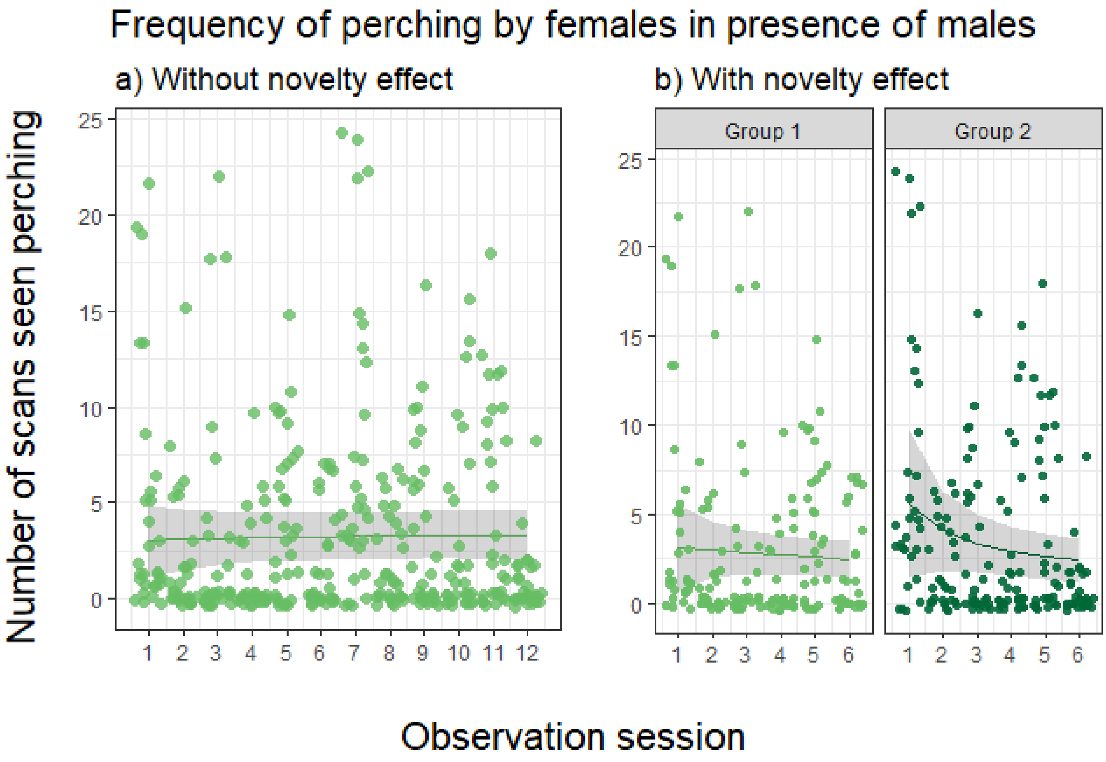


Figure 8: *Perching frequency with respect to experimental stage and observation time. There is a relationship between presence of males and timing of perching (χ^2 = 33.311, df = 2, p < 0.001). When males are not present, perching is much more frequent in morning observation sessions. When males are present, however, there is an increase in perching frequency during evening rather than morning sessions.*

Considering only sessions where males were present, perching significantly declined over successive sessions with each group of males (χ^2 = 14.022, df = 4, p = 0.007). No significant difference was detected between the patterns with the first and second group of males (χ^2 = 7.927, df = 4, p = 0.094).

There was a significant novelty effect: perching is better explained as a function of time with a given group of males than time since first exposure to males (χ^2 = 15.835, df = 4, p = 0.003). No significant slope was detected when perching was modelled as a function of time since first exposure (χ^2 = 0.796, df = 1, p = 0.373). See ***Figure 9*** for a comparison of these two models.

Figure 9: *Frequency of female perching over the course of the experiment (only sessions with males present). Each data point represents the number of scans (out of 24) where a single female was seen perching during a given observation session. Model* ***b)*** *has significantly higher likelihood (χ^2 = 15.835, df = 4, p = 0.003).*



Effects of age and dominance status

Female age had a significant effect on copulation rate and the number of forced copulation attempts received: younger females received significantly more forced copulation attempts and copulated significantly more than either old or middle-aged females. Old females, on the other hand, perched significantly more frequently than young females. In results for perching, the comparison is only between old and young females, because no middle-aged females were used in 2021 replicates.

Male dominance also had significant effects on behavioural outcomes. More dominant males made more unforced copulation attempts. Females were more likely to accept forced attempts from dominant males, but less likely to accept unforced attempts.

No significant effects were detected of female dominance. All test statistics for effects of male and female dominance and female age can be seen in ***Table 5***.

Table 5: Effects of age and dominance status on variables measured. Test statistics refer to likelihood ratio tests between full models (second column in ***Table 4***) and reduced models with the variables concerned removed. Because female age and dominance status are closely associated, they were first tested by removing both from models. If a significant effect of both variables was found, then they were tested individually.

	Effect of female age and dominance together	**Effect of female age alone**	**Effect of female dominance alone**	**Effect of male dominance**
Total number of copulations	Significant effect: χ^2 = 8.559, df = 3, p = 0.036	Young females copulate more than old (d = 0.160) and middle aged (d = 0.247) females (χ^2 = 6.905, df = 2, p = 0.032)	n.s. (χ^2 = 1.298, df = 1, p = 0.255)	n.s. (χ^2 = 3.567, df = 1, p = 0.059)
Number of solicitations by females (negative binomial model)	n.s. (χ^2 = 2.659, df = 3, p = 0.447)	-	-	n.s. (chi = 0.307, df = 1, p = 0.579)
Number of solicitations (logistic model)	n.s. (χ^2 = 2.305, df = 3, p = 0.512)	-	-	n.s. (chi = 0.378, df – 1, p = 0.539)
Number of unforced attempts by males	n.s. (χ^2 = 2.746, df = 3, p = 0.433)	-	-	Dominance associated with significantly increased attempts (χ^2 = 11.335, df = 1, p < 0.001)
Female acceptance rate of unforced attempts	n.s. (χ^2 = 5.911, df = 3, p = 0.116)	-	-	Females significantly less likely to accept attempts from more dominant

				males (χ^2 = 4.296, df = 1, p = 0.038)
Number of forced attempts by males	Significant effect: (χ^2 = 14.982, df = 3, p = 0.002)	Young females receive more forced attempts than old (d = 0.303) and middle-aged females (d = 0.319) (χ^2 = 12.563, df = 2, p = 0.002)	n.s. (χ^2 = 0.379, df = 1, p = 0.538)	n.s. (χ^2 = 1.546, df = 1, p = 0.214)
Female acceptance rate of forced attempts	n.s. (χ^2 = 3.441, df = 3, p = 0.329)	-	-	Females significantly more likely to accept more dominant males (chi = 6.216, df = 1, p = 0.013)
Female perching rate (sessions with males present)	Significant effect (χ^2 = 14.274, df = 4, p = 0.006)	Old females perch more than young females (d = 0.207) (χ^2 = 10.282, df = 2, p = 0.006)	n.s. (χ^2 = 1.473, df = 2, p = 0.479)	N/A (males do not feature in this model)
Female perching rate (sessions with and without males)	Significant effect (χ^2 = 23.458, df = 4, p < 0.001)	Old females perch more than young females (d = 0.106) (χ^2 = 18.096, df = 2, p < 0.001)	n.s. (χ^2 = 0.448, df = 2, p = 0.799)	N/A (males do not feature in this model)

Discussion

Summary of findings

This study set out to investigate the effect of partner novelty on four measures of female sexual receptivity- three direct measures quantifying receptivity in sexual interactions, plus the rate of perching- and to identify any differences associated with female age or social status. We found that female copulation propensity, as quantified by three behavioural measures (number of solicitations, and acceptance rate of forced and non-forced copulation attempts), decreased over time since exposure to males. Some revival of female copulation propensity when supplied with novel males, i.e. a Coolidge-like effect, was found in one measure (solicitations), but not the other two. We found evidence that, despite a high level of male sexual coercion, female behaviour influences copulation rate: despite similar patterns of attempts from males in both experimental groups, the number of successful copulations was significantly higher for the first group of males than the second, suggesting female influence. As well as via resistance and solicitation, we found evidence for females modifying space use as a means of controlling copulation frequency. Females perched more when males were present, and perching rate was highest when males were first introduced, which is when males make the forced attempts. The daily pattern of female perching was also altered when males were present to favour more perching in the evening, when the number of copulation attempts from males was highest.

Context: male behaviour

Broad patterns of male behaviour were remarkably consistent between groups (see ***Figure 3***). The number of attempts at forced copulation were highest when a group of males was first introduced, before declining steeply over successive sessions. This is an interaction type which indicates high motivation to mate in males, since it requires high effort, has no guarantee of success, and may provoke attacks from other males. This pattern is therefore consistent with the well-characterised pattern for males of this species of declining copulation propensity with familiar females (Alvarez-Fernandez et al. 2019; Pizzari et al. 2003). It is possible that male behaviour could have varied between groups one and two according to some cue of females' previously mated status. However, there was no significant difference detected in the intercept or slope between groups one and two, suggesting that males' initial copulation motivation and decline over time was the same for both groups.

The number of unforced copulation attempts, by contrast, was relatively flat over successive observation sessions. There was a slight increase over successive sessions for the second group of males, which does not seem to have a clear explanation. However, the absolute number of attempts did not differ between the first and second groups.

Changing patterns of male copulation attempts could be related to copulation propensity in males as a result of some cue that alerts the second group of males to the recent presence of the first group, indicating that females are likely to have been previously inseminated. However, the fact that the pattern seen in forced attempts does not differ between the first and second groups strongly indicates that any difference in male copulation propensity is minimal. The frequency of unforced approaches is highly influenced by the behaviour of females, since these mostly occur when females choose to spatially associate with males rather than avoid them. We should not conclude, therefore, that changing frequencies are explained solely by male behaviour – they are likely explained by a combination of female and male influences. Further data on the level of female spatial avoidance of males would be required in order to understand this process.

How do females control copulation frequency?

For both groups of males, the highest frequency of successful copulations occurred in the first session where males were introduced. However, the peak copulation frequency was lower for the second group compared to the first, and the total number across all sessions was also lower (***Figure 4***). Since the number of attempts was the same or higher for the second group, this reduction in successful copulations strongly indicates a role of female influence over copulation frequency. Females alter their number of solicitations and the acceptance rate of attempts between groups, showing a lower number of solicitations, a lower probability of solicitation, and a lower acceptance rate of forced and unforced copulation attempts with the second group of males compared to the first.

The soliciting of copulations is the only means by which females can actively, rather than passively, exercise mate choice, by approaching specific males. Unlike the other female behaviours studied, this is a way to increase, rather than decrease, copulation frequency as opposed to simply passively accepting all attempts initiated by males. In this experiment, as is commonly found in this species, rates of solicitation were very low (Pizzari, Froman, and Birkhead 2002; Pizzari and McDonald 2019). They represented less than five percent of sexual interactions recorded, and the predicted number of solicitations from the average female to the average male ranged from zero to 0.09 across observation sessions. Nevertheless, we find that the number of solicitations is highest when the first group of males is added, decreasing steeply over successive sessions, and that this pattern of decline shows a small but significant disruption with an increase in solicitation number when unfamiliar males are added. The same result was found when considering a binary probability of soliciting copulation at least once versus not at all. This increase following a decline in receptivity is suggestive of a pattern akin to a female Coolidge effect. However, since a larger fraction of copulations were forced, assuming this result is robust it would affect overall mating patterns only to a small degree.

Second, we looked at the acceptance rate of unforced copulation attempts. Females resist these attempts by simply stepping away from an approaching male, rather than crouching to allow mounting. This form of resistance is very low-cost and highly effective: the attempt is almost always terminated when the female steps away. As such, when females do accept this form of attempt, it is likely to reflect a ‘genuine’ intention to copulate, rather than avoiding costs of resistance.

As with the number of solicitations, the acceptance rate for unforced copulation attempts was also highest when males were first introduced, before declining over subsequent observation sessions. No Coolidge effect was seen for this type of interaction: adding novel males did not have any significant effect on this trajectory; acceptance rate remained flat throughout the second group of males, with no initial peak. However, this is consistent with a weak Coolidge effect: if females are motivated to seek copulations with sexually novel males, we would expect this to be seen primarily in their pattern of solicitations, where they can exercise a high level of mate choice, and less so in male-initiated attempts (both unforced and forced). Females may derive benefits of polyandry by a small increase in the number of solicitations when novel males are added, with no need to then accept more copulations via other means.

Unforced copulation attempts usually take place when females are socially associating with males; more dominant males were significantly more likely to make these attempts, which may reflect a female social preference for dominant males. By contrast, the acceptance rate for these attempts was higher for lower ranking males. It seems that female mate choice regarding this type of interaction occurs in large part before the accept/reject decision point, so this favouring of lower ranking males almost certainly does not tell the whole story. One explanation could be sexual conflict between high-ranking males, who attempt to monopolise females, and females who seek out covert copulations with a wider range of males. Under this interpretation, social association of females with dominant males is the status quo, during which many unforced approaches occur and may be rejected by females; unforced approaches by subordinate males, however, would have to occur during brief periods of deliberate association by females, contra to the status quo. Again, more information on the spatial associations of these birds is key to understanding these dynamics

Third, we looked at acceptance rate for forced copulation attempts. The pattern here is very similar to the pattern for acceptance of unforced attempts. The acceptance rate was highest when the first group of males are introduced, before declining over successive sessions, though the decline was less steep than for unforced attempts. As with unforced attempts, no Coolidge effect was seen for this type of interaction: the addition of new males does not significantly disrupt the pattern of overall decline in receptivity as quantified by this metric. Again, however, this is consistent with a weak Coolidge effect being seen in the number of solicitations.

Females have little control over this type of interaction. Unlike with unforced attempts, they cannot control which males attempt forced copulations via preferential social association. The only way they can reduce the number of attempted forced copulations they are subject to is by hiding and perching, but this is imprecise (prevents all males equally) and costly (prevents females engaging in other activities).

Resisting attempts at forced copulation when they do occur is not fully effective, and is costly, as it may prolong aggression from the harassing male and may attract aggression from others. It therefore seems likely that acceptance of this type of copulation attempt largely reflects convenience polyandry, ie avoiding costs of resistance, rather than benefits of copulation. We would expect the benefits of convenience polyandry to be highest when males are first introduced and have the greatest motivation to harass females. This factor alone could produce a Coolidge-like effect, with females accepting fewer attempts over successive sessions as male sexual motivation decreases and then increasing again when fresh males are added. If this factor was the only significant driver of female behaviour, then we would expect to see a Coolidge-like effect in this measure and not in solicitations or acceptance of unforced approaches, which are not associated with the same resistance costs. The fact that we do not see an increase in acceptance rate when the second group of males is introduced (with just as high an initial rate of forced attempts as the first group) is surprising, therefore, and may suggest that there are strong costs of excess copulation that outweigh the costs of resistance.

Since resisting attempts from subordinate males sometimes prompts intervention by dominants, resistance is likely to be more effective against subordinate males. The fact that we see a significantly higher acceptance rate for dominant males might therefore reflect the fact that resistance is more worthwhile against subordinate males, rather than reflecting a preference for dominant males. On the other hand, this intervention sometimes results in the dominant male taking the place of the subordinate, so resisting subordinate males may represent a form of mate choice- an 'unwanted' copulation from a dominant male being preferable to one from a subordinate- more than a way to reduce the number of copulations that occur.

Perching as a means for females to avoid harassment by males

In the second half of this experiment (observations carried out in 2021 field season), perching was also investigated as a measure of female resistance to male harassment. When females are on perches, it is very difficult (though not impossible) for males to attempt to copulate with them, so the level of harassment they are subject to, as well as the number of copulations, is much reduced. We find that there is no significant difference in the overall frequency of perching between groups, but a significant trend within groups, whereby the most perching occurs when males are first introduced, coinciding with the highest intensity of harassment.

Spatial avoidance is a major way for females to avoid costs of copulation and sexual harassment. In broilers and broiler breeders flocks, the provision of cover panels has been found to reduce levels of both general and sexual aggression (Cornetto, Estevez, and Douglass 2002; Estevez et al. 2010; Leone and Estévez 2008; Somparn, Damnoensakunchai, and Sutthiluk 2019). Previous research in junglefowl has assumed that perching by females represents an avoidance of male harassment (most notably McDonald et al. 2019). This chapter provides empirical evidence backing this assumption. Comparing the frequency of perching by females during the experimental stages where males were present to the frequency before any males are added, we find that significantly more perching occurs in the presence of males.

Further support for this idea comes from the fact that there is a significant interaction effect between time of day (AM vs PM observations) and the presence or absence of males. When males are absent, females perch more often in the morning, with very little perching in the evening- a time females typically spend engaging in foraging and dust bathing. When males are present, this pattern is reversed, with females spending around twice as much time perching in the evening as in the morning. Since the evening sessions were when most copulation attempts were observed (a result consistent with previous work e.g. Løvlie and Pizzari (2007)), this time-specific adjustment of perching frequency in the presence of males strongly supports the idea that perching functions as a way of avoiding sexual harassment and preventing forced copulations.

In addition, this reversal of daily patterns suggests that females pay a cost to avoid males in this way: we have data showing when they ‘prefer’ to perch when in a single-sex group, and it seems the presence of males causes them to deviate from this pattern. Additional perching is likely to be costly because when perched, females cannot forage to meet their nutritional requirements, or engage in other activities such as dust bathing or nesting. This is consistent with a body of research across several species suggesting that females pay substantial costs to spatially avoid males, trading off harassment intensity against foraging efficiency or predation risk (Darden and Croft 2008; Krupa, Leopold, and Sih 1990; Pilastro, Benetton, and Bisazza 2003; Shine et al. 2005).

Perching is a broad form of resistance as it restricts the number of copulation attempts from all males, as opposed to directly resisting in a particular interaction. The fact that females do this suggests that they benefit by reducing the absolute number of copulations, and the fact that they pay a potentially substantial cost to do so suggests that the cost of excess copulations can be very significant.

What can we conclude about costs and benefits of copulation for female junglefowl?

The way in which copulation propensity responds to sexual novelty can give us an idea of the selection pressures acting on a species’ copulation rates, revealing the relative strengths of costs and benefits of copulation that occur per instance and per partner. In males, we often see a Coolidge effect as a result of two factors. First, a combination of per-copulation costs and per-partner benefits, which means that the payoff of a given number of copulations is generally higher if these copulations are spread over a number of partners than if they are restricted to a single partner. Second, an ecology that means males are sufficiently likely to encounter new partners and remate before they can replenish their sperm reserves. This makes it is worthwhile for a male to hold some ejaculate in reserve, rather than spending it all on the first female he comes across.

In our results, we find strong evidence that there are significant 'per copulation' costs to females, as opposed to costs that are just 'per partner'. Not only do females solicit fewer copulations from familiar males, they are also more likely to resist copulation. Since resistance can be costly, this implies that costs of copulation are of equivalent weight or higher. In addition, the potentially costly alteration of daily perching patterns suggests that male harassment, and possibly also excess matings, have a substantial cost to females that they are willing to go some way to avoid.

We also find evidence suggestive of a significant benefit of copulating with multiple males. In general, females accepting or soliciting copulations may be explained by motivation to acquire an optimum number of copulations (ie, benefits per copulation), by motivation to copulate with certain males (benefits per partner), or by convenience polyandry (avoiding costs of resistance).

Since females in this experiment were subject to intense sexual harassment meaning they were unlikely to be copulating at less than their optimum rate, solicitations are likely to be motivated by benefits of polyandry rather than by achieving a certain number of copulations. Even when unfamiliar males are added, while females slightly increase their number of solicitations, they still actively reduce their number of copulations: they increase perching rates and do not relax resistance to forced and unforced copulation attempts. If anything, it is possible that the intense sexual harassment experienced by females as a result of the high sex ratio used in this experiment may have decreased the observed magnitude of female preference for polyandry, as they are likely to have been in a highly avoidant state.

If the female Coolidge effect was driven primarily by 'negative' benefits (avoiding costs of resistance), then we might expect to see lower resistance to novel males, who are highly motivated to mate and may impose significant resistance costs on females. The fact that the Coolidge effect is only seen in solicitations suggests that it is driven primarily by 'positive' benefits of polyandry, such as genetic benefits and fertility insurance. Given the high number of forced copulations females were subject to in this experiment, and the high costs females pay to keep this number down, these benefits are likely to be substantial if females are willing to go out of their way to increase their number of copulations by soliciting novel males.

Female dominance and age

Age and social dominance are traits that have extremely significant effects on the social, sexual, and reproductive lives of junglefowl (Carleial, McDonald, and Pizzari 2020; McDonald et al. 2019; Pizzari and McDonald 2019). We expected female responses to sexual novelty to potentially vary according to their age or dominance status. We expect this partially because of inherent changes to optimal outcomes for females, and partially due to factors like differing levels of harassment received from males, which might mean different levels of "compensatory resistance" are required to achieve the same outcomes (see Chapter 1).

An increasing body of recent work in a number of species suggests that female choosiness varies plastically across the life cycle, for instance decreasing as females senesce (Kodric-Brown and Nicoletto 2001; Lynch et al. 2005; Moore and Moore 2001; Perry and Hopkins 2021). Dominance may allow females more options for exercising control over copulation frequency, since dominant females outcompete subordinates for hiding and perching places (Wang et al. 2022). Furthermore, even if optimal strategies are the same, there may be an element of learning through experience that means older females are better able to execute those strategies. For instance, recent work in the same population of captive red junglefowl found that older females formed close social associations and spent more time perching, which reduced harassment from males, and could plausibly represent learned responses that younger, naïve females do not adopt (McDonald et al. 2019).

Younger females and more dominant females are more attractive to males and may receive a larger number of copulation attempts (McDonald et al. 2019; Pizzari et al. 2003). In addition, younger females are more efficient at storing sperm, and therefore may require fewer inseminations to ensure fertility of their eggs (Brillard 1992). All else being equal, therefore, we would expect younger or more dominant females to show lower sexual receptivity in order to achieve the same number of copulations, and to be more selective in their receptivity, since they would have a wider range of partners to choose from. Dominant status strongly correlated with older age in our study, however, so any effects of age or dominance arising from different levels of male interest may cancel each other out.

Testing for effects of female age or dominance was a secondary aim of this chapter. We found a significant difference only in the number of forced attempts received by age, with younger females receiving significantly more of this type of attempt than old or middle-aged females, as well as a higher overall copulation rate. Solicitation and acceptance rates did not vary according to female age or dominance status. The only female behaviour that did vary according to female characteristics was the frequency of perching, with older females perching significantly more than younger females. This may be a tactic females learn with age to avoid forced copulations. Alternatively, the higher proportion of time spent perching by older females might contribute to explaining why they copulate less and receive fewer forced copulations, even if they perch for unrelated reasons.

Future directions

The perspectives provided by this study are limited by the fact that only one group size, one sex ratio and one set of timings was used. Altering the sex ratio would allow us to manipulate the level of sexual harassment (more male-biased sex ratio means more harassment suffered by any one female). This would allow us to investigate how the costs of excess copulations and benefits of polyandry trade off against each other. For instance, if the rate of sexual harassment and the number of forced copulations females were subject to had been higher, we might expect not to see a signal of a Coolidge effect, because the pressure to keep copulations to a minimum might have outweighed benefits of polyandry.

On the other hand, if only one male rather than three had been available in the first group of males, we might have seen a stronger Coolidge signal when a novel male or males were provided, as the diversity of sperm already acquired would have been much lower. We could also test the robustness of conclusions drawn here by trying alternative structures, such as leaving a longer gap between the removal of old males and the addition of new ones, or the addition of a third group of males after the second.

In addition, these conclusions are limited by the fact that our analysis considers how behavioural measures vary over successive observation sessions, rather than over successive copulations. The latter analysis would be more complex to execute, but might correct for some assumptions that are made for the first analysis. For instance, if one female mates twice as frequently as another, then we might not expect their behaviour to change over time at the same rate.

Conclusion

This chapter provides a novel insight into female responses to the novelty of sexual partners in this species. Female receptivity showed an exponential decline over time since exposure to males across multiple measures. The patterns of daily perching behaviour observed in this chapter suggest that females engage in resistance despite this being costly. In addition, females show a slightly increased propensity to solicit copulations from novel males, but they did not show an increased propensity to accept either forced or unforced copulation attempts. Taken together, these results illuminate the fitness costs and benefits to females of polyandry, suggesting that polyandry is driven in part by benefits to females, and not just by the avoidance of costs.

Chapter 3: Opportunity for selection in broiler breeder males with and without male intrasexual competition

Everything can be inherited except infertility.

- *Matt Ridley,* The Red Queen (1993)

Abstract

Opportunity for selection, *I*, is the standardised variance in reproductive success within a population, and is equal to the largest possible selection differential on any trait. By partitioning variance in reproductive success arising from different sources, it can also give the largest possible selection differential on different components of reproductive success (e.g. mating success, paternity share, and mate fecundity). Variance in mating success is frequently described as the "opportunity for sexual selection", I_S. However, sexual selection via other components of success will be missed by this measure. This chapter investigates what is missed by using I_S as a measure of sexual selection. We calculate the opportunity for selection in populations of broiler breeder males with and without male intrasexual competition, and investigate the effect intrasexual competition has on different components of reproductive success. We find that mating success is by far the largest contributor to variance in reproductive success, both in the with and without competition treatments, with paternity share contributing a little in the competition treatment, and mate fecundity contributing a near-negligible amount in both treatments. We also examine whether any behavioural measures are associated with reproductive success on an individual level, and do not find any association.

Contributions

This chapter was conceived by Tommaso Pizzari. Behavioural data was collected by Ellen Pasternack. Parentage data was collected in raw form by Aviagen and processed by Ellen Pasternack. Analysis was carried out by Ellen Pasternack, with input from Tommaso Pizzari, Grant McDonald, and Eleanor Bath. The chapter was written by Ellen Pasternack, with input from Grant McDonald, Eleanor Bath, and Tommaso Pizzari.

Introduction

Quantifying the opportunity for selection: the importance of variability in reproductive success

Individuals within a population can vary greatly in the number of offspring they produce (Clutton-Brock 1988). It is this variation that allows natural and sexual selection to occur. The core idea of evolution by natural selection is that heritable traits logically *must* increase or decrease in relative frequency over successive generations as a result of their impact on an individual's reproductive output. There are three elements to this process: (i) individuals vary in some heritable trait, (ii) individuals vary the number of viable offspring produced (i.e. lifetime reproductive success), and (iii) variation in lifetime reproductive success can be explained to some degree as function of variation in heritable traits. These elements (i)-(iii) are all essential for selection (Kingsolver and Pfennig 2007).

Researchers studying the operation of natural selection work to quantify each of these elements in the selective process (Kingsolver and Pfennig 2007; Wood and Brodie 2016). Firstly, what is the distribution of traits, and how much of the variation that we see is heritable? Secondly, what is the nature of the link between heritable traits and fitness: is there a linear or more complex polynomial relationship? Is fitness higher for individuals at one end of the trait distribution (directional selection), in the middle (stabilising selection), or at both extremes (disruptive selection)? And thirdly, what is the distribution of individual fitnesses to which traits may be linked?

The relationship between a trait and individual fitness can be described by the selection differential. The selection differential of a trait is the covariance between that trait and fitness (Arnold and Wade 1984). It also relates to the rate of change on that trait due to selection. It is equal to the difference between the population mean trait value in one generation and the mean trait value for the subset of the population who are the parents of the next generation. Multiplying this by the trait's narrow-sense heritability gives the expected change in the population mean value of a trait from one generation to the next – this relationship is known as the Breeder's Equation (Jones 2009; Kelly 2011).

Because covariance is a statistical measure of the joint variability between two variables x and y, consisting of the mean value of $(x - \bar{x})(y - \bar{y})$ across all data points (x,y) in the population. If there is no variation in fitness, then covariance can only equal zero: for every data point (x,y), the fitness y will be equal to $\bar{y}$ and so $(y - \bar{y})$ will always equal zero. Therefore, in the absence of any individual variation in fitness (or equally, in the absence of individual variation in the phenotypic trait), the only possible selection differential is zero: selection cannot occur on any trait.

The measure of fitness most commonly favoured is lifetime reproductive success (the total number of offspring produced in an individual's lifetime), as it is conceptually simple and combines well with quantitative genetic theory. This measure is not perfect, however: for instance, it does not account for offspring quality or survival, which may cause significant deviations from the conceptual ideal of fitness as an individual's genetic contribution to future generations (Brommer et al. 2004; Young et al. 2022). Additionally, data gathering may be prohibitively difficult for long-lived species. Alternatives such as lifetime number of fledglings produced or number of offspring produced in a single reproductive event may be used for either conceptual or practical reasons (Bonnet et al. 2022; Brommer et al. 2004; Clutton-Brock 1988; Grafen 1988).

The extent of variation in reproductive success is quantified by researchers using the measure opportunity for selection (*I*), which is the standardised variance in reproductive success, that is, variance in reproductive success divided by the square of its mean within a population (Arnold and Wade 1984; Crow 1958). *I* is effectively the covariance of reproductive success with itself, and thus represents the maximum possible selection differential for any trait within that population: if a trait had a one-to-one correlation with reproductive success, then its selection differential would be equal to *I*. This measure is sometimes called the "index of selection" or "intensity of selection", but this is misleading because a high value of *I* does not necessarily indicate that strong selection is occurring unless some trait is strongly linked to that large amount of variation in fitness. We expect a certain amount of variation in reproductive success to be the result of stochasticity and not associated with trait variation. It is more accurate to refer to *I* as the "opportunity for selection": an upper limit on the possible strength of selection that may occur in a population (Grafen 1988; H Klug et al. 2010; Krakauer et al. 2011).

The standardisation of dividing by the square of the mean makes *I* a unitless measure that may be compared across populations. The minimum value *I* can take is 0; values above around 1 (as seen for instance by McDonald *et al.* (2017) among male red junglefowl) are moderately high; while the values of around 10 observed in a lekking bird species by DuVal and Kempenaers (2008) are extremely high.

Opportunity for sexual selection

I- the total opportunity for selection- can be partitioned into variance arising from different sources of variation in reproductive success, to reveal the largest possible selection differential for traits associated with certain episodes of selection (Anthes et al. 2010; Crow 1958; Grafen 1988; Jones, Arguello, and Arnold 2002). This is illustrated for instance by (Webster et al. 1995), who separately calculate variance in the number of chicks produced via extra-pair and within-pair fertilisations for male red-winged blackbirds *Agelaius phoeniceus*. The variance in the number of extra-pair chicks is the largest possible direct selection differential for traits with the sole effect of helping males to obtain extra-pair paternity (the *opportunity for selection via* extra-pair paternity); likewise, the variance in number of within-pair chicks gives the opportunity for selection via within-pair paternity.

The opportunity for sexual selection represents a special case of partitioning total selection. The standardised variance in mating success (number of mates acquired over a lifetime, a breeding season, or some other predefined period) is often given as an estimate of the opportunity for sexual selection, and is termed I_s (Jones 2009; Wade 1979). As with I, a high I_s does not mean that there is necessarily a high level of selection occurring. However, a very low or zero I_s (all individuals have the same number of mating partners) would mean that the acquisition of mates could not be under direct selection, since there is insufficient variation in number of mates acquired. This quantitative measure allows us to compare potential strength of selection across different situations. In particular, many studies compare I_s between the sexes, generally finding that the opportunity for selection via competition for mates is higher in males than females (Brown, Laland, and Borgerhoff Mulder 2009; Janicke et al. 2016), although Levine *et al.* (2020) find no difference between the sexes in this measure.

While I_s is a convenient measure, it does not match with what we understand sexual selection to mean. A 2010 review found that I_s was the most commonly used measure of sexual selection, and that many authors were not clear about its shortcomings, claiming for instance that I_s reflects the ”strength of sexual selection” when in fact it represents an *upper limit* for the strength of *selection via mate number*, and we have very little idea how close to that limit the true strength of sexual selection may be (Klug et al. 2010). Sexual selection is defined as selection arising from competition for access to the gametes and reproductive investment of the opposite sex (Darwin 1871; G. A. Parker 1970; Geoffrey A Parker 1982). The total opportunity for selection is the largest possible selection differential of any trait, and the opportunity for *sexual* selection is the largest possible *sexual* selection differential (Jones 2009). Properly, then, this ought to be the variance in reproductive success that is attributable to sexual competition. Instead, I_s captures the opportunity for selection due to individuals having different numbers of mating partners. High variance in number of mating partners may often be associated to high levels of sexual competition, but they are conceptually distinct.

Here, I outline the two key problems with I_s as a measure of opportunity for sexual selection: firstly, competition for mating partners is not the only episode of sexual selection, and secondly, not all inter-individual variation in the number of mating partners is variation on which sexual selection can act. Next, I propose a framework that can help to overcome these sources of error. This chapter will then apply this framework in a population of broiler breeder males.

Problem 1: I_s doesn’t capture all episodes of sexual selection

The total reproductive success of males, T, can be conceptualised as the product of several sources of variance, plus random error (see ***Figure 1***). Males vary in the number of mating partners they acquire (mate number, M), the mean fecundity of those partners, or in other words, the number of potential embryos available to be sired (N), and their paternity share- the proportion of their partners' embryos that they sire (P) (Collet et al. 2012; McDonald et al. 2017; Webster et al. 1995). Competition between males for access to reproductive opportunities has the potential to increase variance in all three of these multiplicative components, as males compete for the highest fecundity mates (those with high N) and strive to increase paternity share (P) in sperm competition.

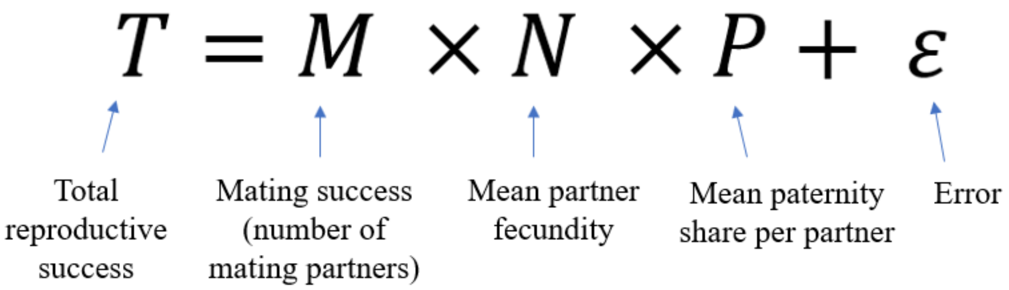


Figure 1: *Decomposition of total male reproductive success into three multiplicative sources of variance, plus error* (Collet et al. 2012; McDonald et al. 2017; Webster et al. 1995).

I_s is equal to variance in the first component, M, and does not capture any of the other components. Labelling this quantity as I_s instead of simply var(M) is arguably misleading as it elevates M over the other components of success and implies that variance in this component is equal to the opportunity for sexual selection, when in fact mathematically M, N and P have equal weight in determining reproductive success.

Put another way, variance in the number of unique mates is only of interest as a measure of selection insofar as it links to the total number of offspring, as reflected by the Bateman gradient, the slope of the regression of total reproductive success (T) on mate number, or, equivalently, the selection gradient on mate number (M) (Anthes et al. 2010; Jones 2009). M is not in itself something that selection operates on; rather, the offspring produced with those mates is. Equally, variance in N or P is only relevant to the extent that it feeds into variance in T. To illustrate with an extreme example, if we had high variance in M (so high I_s), but through post-copulatory processes this translated to equal numbers of offspring produced for every individual, then the true opportunity for sexual selection would be zero. Conversely, if there was no variance in M (so $I_s = 0$), but high variance in P or N, then in fact the true opportunity for sexual selection would be greater than zero.

The fact that I_s ignores variance in mate fecundity (N) may cause significant error in using it as an exclusive measure of the opportunity for sexual selection. Competition for access to or monopolisation of high quality mates is likely to be an especially significant aspect of sexual selection for females, for whom mate quantity is less closely tied to reproductive success (Fitzpatrick 2015; Rosvall 2011), but can also be relevant for males (McDonald et al. 2017; Webster et al. 1995).

Similarly, the fact that I_s ignores variance in paternity share (P) means that post-copulatory selection is not captured when I_s is used as a measure of opportunity for sexual selection. This would be less of a problem if we knew that success pre- and post-copulatory episodes of competition were strongly positively correlated. However, this is not necessarily the case: often male investment in pre- and post-copulatory sexual selection may trade off against each other (Cornwallis and Birkhead 2007a; Kahrl, Cox, and Cox 2016; Parker and Pizzari 2010; Pizzari, Cornwallis, and Froman 2007; Simmons, Lüpold, and Fitzpatrick 2017; Warner et al. 1995) or vary independently (Pélissié et al. 2014). We also know that post-copulatory competition can be a critical determinant of individual reproductive success, and in some mating systems characterised by high polyandry, post-copulatory competition can have more weight than pre-copulatory competition (Marie-Orleach et al. 2016; McDonald et al. 2017). We cannot therefore assume that the magnitude of this error will be small.

Problem 2: hidden sources of variance

Non-competitive sources of variance

We expect variation in phenotypes to result in differences in the ability of individuals to copulate or achieve fertilisation even in the absence of competition. For instance, we cannot attribute the low number of genetic mating partners of an individual that is totally sterile to intrasexual competition, because this outcome would have been the same with or without competition. Recalling that sexual selection is selection that occurs *as a result of competition for mating opportunities*, this sterility cannot be subject to sexual selection (Darwin 1871; West-Eberhard 2014). Sterility is subject to “hard selection” (Wallace 1975): a sterile individual cannot reproduce, no matter how much or how little reproductive success others in the population attain. Sexual selection is by definition a form of “soft selection”, whereby individual fitness can only be considered relative to competitors.

Sterility is an extreme example, but there are many other traits which have the potential to affect individuals’ abilities to find, attract, and monopolise mates, and to achieve fertilisation success post-copulation, in a similarly “hard selected” way- i.e. not as an outcome of competition. Such traits are expected to contribute to variance in reproductive success within a population.

These innate, non-competitive fitness differences may be correlated with success in competition for mating opportunities, as individuals that are generally fitter may both do better in intrasexual competition as well as having better innate reproductive ability. Alternatively, they may be uncorrelated. In either case, not accounting for non-competitive differences in sexual fitness will cause estimates of opportunity for sexual selection to be systematically inflated.

Some authors consider that non-competitive variance can also be included in the definition of sexual selection (Shuker and Kvarnemo 2021). For instance, (Murphy 1998) conceptualises a divide between “interaction-dependent” and “interaction-independent” sexual selection, with the latter being selection arising from what I have here described as “non-competitive sources of variance”. Nevertheless, whether we consider this form of selection to lie within or outside the definition of sexual selection, there is a clear conceptual distinction between interaction-dependent and interaction-independent selection, and it would be of scientific interest to know what the relative opportunity for selection is through each.

Non-phenotypic sources of variance

Even if individuals do not differ in innate reproductive ability, we would still not expect uniform mating or fertilisation success in the absence of competition. As previously discussed, male reproductive success is considered to be a product of the number of females he mates with, his fertilisation success with each of these females, and each female’s fecundity (Collet et al. 2012). None of these components is under total phenotypic control of the male: there will be some residual variance in each. Similar could be said for females: a female’s reproductive output, in the absence of intrasexual competition, depends on both her own phenotype, and the phenotypes of males she mates with.

The potential for random chance to create the illusion of “better” and “worse” performing individuals has been illustrated by both theoretical and empirical work (M. D. Jennions, Kokko, and Klug 2012; H Klug et al. 2010; Hope Klug and Stone 2021; Sutherland 1985). Environmental variation, which may be random with respect to individual phenotype, can strongly influence an individual’s reproductive success (Banks and Thompson 1985), or even create the illusion of correlation between a phenotypic trait, such as large body size, with reproductive success, when in reality both large size and high reproductive success are caused by favourable circumstances during development, a phenomenon termed the “silver spoon effect” (Grafen 1988).

In general, the null hypothesis ought to be that variation in reproductive success is random with respect to phenotype, since this is the most parsimonious explanation for some individuals performing better than others. Only when variation cannot be explained by a statistical distribution based on random mating should we consider that it may be caused by individual variation in phenotypic traits.

The “true” opportunity for sexual selection could be conceived as the standardised variance in reproductive success that is *actually caused* by intrasexual competition. Some of this variance will be missed when I_s is used as the measure of opportunity for selection, since I_s is variance in M, yet variance in N and P are also expected to contribute. Additionally, some proportion of the variance included in I_s would have been present regardless of intra-sexual competition and thus should not be considered “true” opportunity for selection. The magnitude of this pre-existing variance is not well understood, and so we have little indication of how well I_s tracks with the true opportunity for selection.

The “true” opportunity for selection

Despite its shortcomings, I_s is a valuable metric that can inform us about the circumstances in which one component of selection (selection via mate number) operates. However, using it as a measure of opportunity for sexual selection is flawed, and is likely to produce systematically biased estimates. The true opportunity for sexual selection would need to:

> **a)** exclude variance attributable to mechanisms other than intra-sexual competition, i.e. stochastic variance and variance due to non-social traits, such as sterility; and
>
> **b)** include sexual selection via N and P.

Without reliable estimates of these sources of variation, we do not know the extent to which I_s is likely to be biased, and the true opportunity for sexual selection remains unknown.

This chapter aims to investigate what is missed by using I_s as a measure of the opportunity for sexual selection, using a population of pedigree broiler breeders.

Our study system, broiler breeders

This study uses broiler breeders, a lineage of domestic chickens that have been bred for large size, rapid growth, and high reproductive output. Domestic chickens are in turn descended from wild junglefowl, primarily red junglefowl. Red junglefowl, domestic chickens, and broiler breeders are all members of the same species, *Gallus gallus*, referred to generally in this thesis as “fowl”.

Broiler breeders are under very intense artificial selection, since individuals selected to contribute to future generations have the chance to produce hundreds of offspring that will feed back into the pedigree, while others do not. However, this artificial selection acts on top of naturally occurring selection, since individuals are expected to have greater or lesser reproductive success within a commercial flock even without the interference of breeders selecting for desired outcomes. Understanding this naturally occurring selection is of commercial relevance, because it very likely influences the outcomes of artificial selection, whether by acting in the same or opposite directions. In addition, the poultry industry has an interest in knowing what makes broiler breeders more reproductively successful- whether the answer is certain traits, behaviours, or just random chance- in order to maximise the efficiency of production.

This species is one in which there are strongly skewed distribution of reproductive success. Early work on domestic chickens found substantial inter-individual variation in reproductive success between males (Guhl and Warren 1946). Similarly, in their seven year study of red junglefowl at San Diego zoo, Collias and Collias (1996) found that the distribution of reproductive success was such that the number of adults (of both sexes) making a genetic contribution to future generations was only 28% of what it would be if progeny were distributed randomly.

Differential reproductive success in male fowl can occur via three multiplicative sources of variance: mating success, paternity success, and partner fecundity (***Figure 1***).

Fowl are a polygynandrous mating system, where there are no pair bonds and females generally mate multiply, giving a large potential for variance in mating success among males. Early work in domestic chickens showed very clearly that dominant males copulate with more mating partners (Craig, Ortman, and Guhl 1965; Guhl, Collias, and Allee 1945; Guhl and Warren 1946). This variance can be very large, with lower ranking males frequently being almost completely excluded from mating opportunities. More recent work in junglefowl has also found a strong association between social dominance in males and mating success (Carleial, McDonald, and Pizzari 2020; McDonald et al. 2017; Roth et al. 2021).

There are two main mechanisms that cause this. Firstly, females preferentially solicit copulations with dominant males, and are more likely to resist copulations with subordinate males (Johnsen, Zuk and Fessler, 2001; Løvlie, Zidar and Berneheim, 2014; Roth *et al.*, 2021). Secondly, dominant males monopolise access to females, aggressively interrupting subordinates when they attempt to court or copulate with females (Løvlie, Zidar, and Berneheim 2014; Pizzari 2001). The aggression subordinate males suffer can be intense, and sometimes they appear to give up on copulation as a result, becoming, in the words of Guhl, Collias, and Allee (1945), “psychologically castrated”: they will not resume mating behaviour even when the dominant male, and therefore the threat of retaliation, is removed.

Secondly, when females are polyandrous, males may attain different paternity shares, meaning that two males who have the same number of mating partners may sire different numbers of chicks. In this species, with prolonged sperm storage and a single ejaculate potentially siring a dozen or more eggs over successive days, there is very large potential for variation. A large determinant of variation will be the level of sperm competition, which will vary according to the level of female polyandry, both from population to population and between females within a single population (Pizzari and McDonald 2019). In addition, individual males vary in the competitiveness of their ejaculates (Bilcik, Estevez, and Russek-Cohen 2005; Bowling et al. 2003; McDonald et al. 2017; Robertson et al. 1998). Females may further bias paternity share via sperm ejection at the time of copulation (Dean, Nakagawa, and Pizzari 2011; Pizzari and Birkhead 2000) or via unknown mechanisms of cryptic female choice at some point during the uptake of sperm into storage tubules, its maintenance, or release prior to fertilisation (Firman et al. 2017). Dominant males seem to enjoy greater paternity shares as well as greater success in the pre-copulatory stage (McDonald et al. 2017).

Finally, females vary in their fecundity, so the potential chicks available to be sired by a male will depend on which females he mates with. Female comb size seems to be correlated with fecundity, and male preference for larger-combed females may therefore reflect an adaptive preference for higher fecundity females (Cornwallis and Birkhead 2007b; Pizzari et al. 2003). Dominant females (who tend to have larger combs as well as larger body size) typically have greater ability to acquire resources and rear more chicks in their lifetime (Carleial, McDonald, and Pizzari 2020; Collias, Collias, and Jennrich 1994); they also lay heavier eggs, suggesting greater ability to provision offspring (Cornwallis and Birkhead 2007b; Müller et al. 2002). (Collias and Collias 1996) found that variance in female reproductive success in red junglefowl was surprisingly large, with 14% of hens never being observed to have eggs or chicks. There is therefore plenty of scope for males to vary in their reproductive success via which hens they mate with, even if their number of mating partners and their average paternity share was uniform.

Despite all the sources of variance in male reproductive success that are connected to sexual behaviour and physiology, we do not actually know to what extent they provide opportunity for *sexual selection*- i.e., to what extent they are caused by social competition between males. For example, it is common to make the inference that, because aggressive, dominant males prevent subordinates from mating, male-male competition is responsible for difference in mating success between dominant and subordinate males. However, it might be that low quality males (who are likely to be at the bottom of hierarchies) might have lower mating rates even without competition. They may have lower propensity to mate due to worse condition, lower agility and lower levels of activity. These males' low mating success, then, would *not* be caused by intrasexual competition, and sexual selection would not be able to act on it.

Reproductive success, both in total and via each of its multiplicative components (M, N, and P), will also be subject to stochastic processes, which means that we would not expect uniform distributions even if all males had identical phenotypes and did not experience competition. However, without knowing how much variation we ought to expect due to random chance and due to non-competitive phenotype, we cannot know how much opportunity for sexual selection there truly is in a population. In addition, without knowing how much opportunity for sexual selection there is at each of the multiplicative sources of variance (M, N, and P), we cannot infer which traits might be under sexual selection. This chapter will attempt to address this knowledge gap by establishing estimates for the extent to which intrasexual competition creates variable success in male mating success, paternity share, mate fecundity, and overall reproductive success.

Fowl are strongly sexually dimorphic and ornamented, and show large inter-individual variance in reproductive success and mating success, especially in males. These suggest that sexual selection is acting on this species, or has done so in the recent past. However, other than social dominance, which has been consistently shown to be associated with reproductive success, the evidence for a link between individual traits and reproductive success is mixed and context-dependent, despite a programme of research around 20 years ago that investigated traits such as comb and wattle size, ornamental feathers in males, and even facial symmetry and eye colour (Zuk, Ligon and Thornhill, 1992; Chappell *et al.*, 1997; Johnsen, Zuk and Fessler, 2001; Bilcik, Estevez and Russek-Cohen, 2005). This perplexing lack of a clear signal may be in part caused by the complex social modulation of reproductive success in this species, which may make isolating the effect of any one trait difficult. For instance, (Johnsen, Zuk and Fessler, 2001) report a complex interactive effect between comb size and male and female dominance on female mating preferences.

In taking the trait-agnostic approach of considering *opportunity for selection*, this chapter investigates selection without focus on any single trait. Decomposing variance in reproductive success into its constituent parts, and determining how much variance in each part may be available for sexual selection to operate on, may indicate which sorts of traits may be under sexual selection.

Aims of this chapter

This chapter has four main aims.

First, we will estimate the amount of variance in male reproductive success that is due to stochastic variance and variance due to non-social traits, such as sterility. This will be achieved by comparing the total opportunity for selection, I, on male pedigree broiler breeders under two different husbandry conditions: one in which intra-sexual competition is present (males compete amongst themselves, and females exercise choice), and one in which it is absent (males do not physically interact with each other, and females have access to one male only and so cannot choose between multiple males). As far as possible, other conditions will be kept constant, so that differences in I under the two conditions may be attributed to the presence or absence of male intra-sexual competition. We expect that I will be larger under intra-sexual competition. I under no competition ought to represent variance that does *not* contribute to true opportunity for sexual selection, since it is not attributable to intra-sexual competition between males. The difference between values of I under these two conditions ought, therefore, to represent an estimate of the true opportunity for sexual selection, because it is the proportion of total opportunity for selection that is attributable to intra-sexual competition.

Second, we statistically interpolate what proportion (if any) of each component can be attributed to male identity in the absence of competition, and what proportion can be explained independently of male identity, distinguishing between the two "hidden sources of variance" discussed above: phenotypically-associated variance that is independent of intrasexual competition, and phenotype-independent variance.

Third, we quantify standardised variance in total reproductive success, T (i.e. I), and its multiplicative components: mate number, M (i.e. I_s), mate fecundity, N, and paternity share, P. This experimental setup enables us to examine the relative sizes of these components of variance both in the presence and absence of male intra-sexual competition.

Finally, we study the social behaviour of individual males in an attempt to identify any behavioural phenotypic traits which may associated with variance in reproductive success and as such, may be under either sexual or non-sexual selection.

As far as I am aware, the only previously published estimates for opportunity for selection and its decomposition in this species is McDonald et al.'s (2017) study of red junglefowl. This chapter provides additional information because i) it is in broiler breeders, not junglefowl; ii) it eliminates variation in male age, which explains a large amount of variance in reproductive success in McDonald's study; and most importantly, iii) it is able to separate variance resulting from physical competition from that resulting from intrinsic phenotypic difference and randomness, as previously detailed in this introduction.

Methods

Experimental setup

Broiler breeders under two different husbandry setups on two Aviagen pedigree farms in East Lothian, Scotland, were studied. Two flocks were studied on each farm, all of the same genetic lineage, each flock initially comprising twelve males and 144 females.

At farm A, the line studied were kept in the 'single sire' setup, meaning that flocks were split into twelve separately-housed groups of one male and twelve females. We therefore assume that there is no opportunity for sexual selection on males (since there is no opportunity for males to compete for reproductive opportunities via physical interaction, including sperm competition). Any variation between males is attributed to differences in males' ability to copulate and fertilise the ova of the females in the absence of social competition, combined with differences between the reproductive ability of the individual females they mate with. Auditory and visual competition with neighbouring males via display may also be possible at farm A (see details of setup below).

At farm B, birds of the same pedigree line were kept in the 'bulk' setup, meaning that the entire flock of twelve males and 144 females were housed together. Since males are free to interact with each other in this setup, and females have the potential to exercise mate choice, we expect these to act as additional sources of variation in mating success, on top of the sources of variation for males in the single sire setup. Farm A and Farm B are extremely similar, but at Farm A the opportunity for males to compete for reproductive opportunities is much reduced.

In addition, observations of mating-related behaviours (see ***Table 1***) were made for all four flocks, with the aim of determining whether these could explain variance in reproductive success or mating success on an individual level.

Study population

At the single sire farm, each flock of 144 females and 12 males was divided into 12 sub-flocks, each of 12 females and one male. Each sub-flock was housed in adjacent enclosures of approximately 1.7 x 2.5 metres. Each sub-flock would have visual and acoustic contact with sub-flocks on either side, but no physical interaction was possible. These groupings were maintained for the entirety of the birds' reproductive lives, meaning that all reproduction would take place within them.

At the bulk farm, each flock of 144 females and 12 males was housed in a larger enclosure of approximately 13 x 13 metres. Within each of the two flocks on Farm B, all birds were able to freely interact in a single group.

Visits to the two farms spanned a decision by Aviagen to phase out the single sire setup in favour of the bulk setup across all birds. Thus, individual birds' placement in either setup was independent of identity or phenotype.

At both farms, birds were kept on a 14:10 L:D schedule and fed on broiler breeder feed pellets according to industry guidelines (Corzo and Silva 2020). All birds were fed once daily at around 08:00. At the time when behavioural observations were taken, birds were at "peak production" (mean age around 30 weeks, with a span of 6 weeks from oldest to youngest).

Birds had continuous access to water via ad libitum water drinkers along one wall of their enclosures. Enclosures were furnished with nest boxes around the perimeter, and in the case of "bulk" groups, in the centre, providing a visual barrier breaking up the open space. A substrate of wood shavings was provided.

Parentage data

For the entirety of the birds' reproductive lives, all viable eggs were collected and incubated by Aviagen staff. Eggs were stored for up to three weeks prior to incubation. Eggs laid outside nest boxes were not incubated, but these made up less than 2% of the total. Maternity was assigned using trap nests and the uniquely identifying wingbands of hens: the hen that laid each non-floor egg was able to be identified, and her wingband scanned to generate a barcode for the egg. For single-sire flocks, paternity was known because there was only one potential sire in each sub-flock. For bulk flocks, paternity was assigned by Aviagen via in-house genetic analysis of tissue samples taken from chicks, using customised parental assignment algorithms developed in-house that have thoroughly validated for accuracy. This was all conducted as part of Aviagen's standard husbandry practice for these flocks.

Calculations of opportunity for selection

For all four flocks (two at each at the single sire and bulk farms), the following were calculated:

- I, the total opportunity for selection: standardised variance in number of chicks per male
- I_s, the standardised variance in number of genetic mates per male
- The standardised variance in mean mate fecundity per male (N)

In the bulk farm only, the standardised variance in paternity share (P) per male was also calculated. This was not calculated for the single sire farm because this setup does not allow any variance in paternity share.

Calculations were made based on the parentage data from the first five weeks of eggs to hatch from each flock. The reason for using a five week sample rather than whole lifetime reproductive success was variable mortality. Some of the original twelve males in each group died prematurely (causes of death for individual birds are not known). In addition, if males performed very poorly in terms of chick production, then they were culled and replaced with a different male. Therefore, using lifetime chick production would bias estimates against less successful individuals.

Using the first five weeks of data only, we were able to obtain a sample of all twelve "original" males in both single sire flocks. We were able to obtain first-five-weeks samples that contained eleven "original" males in both bulk mating flocks. In both of the bulk flocks, a twelfth male was present for part of the "first five weeks" sample as a replacement for males who died early. The replacement males were used in these cases (averaged over weeks in which they were present), because the amount of data from the original males was so low and because it was the replacement males that were present when I visited to collect behavioural data.

I, the total standardised variance in reproductive success, was calculated for each flock as the between-male variance in mean weekly reproductive success (number of chicks sired), over the square of the mean. This means that for each male, his mean weekly number of chicks sired (in total across all females) was calculated across the weeks he was present in the five-week sampling period, giving a single figure for each male. The variance in this figure was then calculated across each of the four flocks and divided by the square of the mean of this figure for that flock.

Each male's number of unique mating partners, mean mate fecundity, and paternity share were also calculated based on genetic parentage. A male's mating success was his number of genetic mating partners (i.e. the number of unique females with whom he sired at least one chick). Females were treated as unique from one week to the next: If one male sired chicks with the same ten females week after week, and another male sired chicks with ten new females each week, they would both have a mean weekly mating success of ten. This means that a male's mating success is measured as the number of unique females with whom he produces at least one chick, mate fecundity is the mean fecundity of those genetic mates, and paternity share is the proportion of those genetic mates' chicks which a male sires (Avise et al. 2002; Jones and Ardren 2003; Jones, Arguello, and Arnold 2002). An individual's number of "genetic mating partners" (the number of mating partners with whom an individual produces offspring) will always be equal to or lower than their number of behavioural mating partners (the number of partners with whom an individual copulates), because it is possible for copulations to result in zero offspring, but not vice versa. Whether estimates of variance in M are higher or lower when relying on genetic parentage data depends on how these "hidden" copulations are distributed. However, variance in paternity share is likely to be systematically underestimated, because if a male has zero paternity share with a partner (they copulate but he does not sire any chicks) then this is not included in the data.

Mate fecundity was the mean number of chicks produced across a male's genetic mating partners. Paternity share was the proportion of the total chicks produced by a male's genetic mating partners that were sired by him (i.e. for each male the total number of chicks he sired across all his genetic mates that week divided by the total number of chicks produced by his genetic mates). For single sire males this figure is always 1, and for bulk males it can in theory vary between 0 and 1 - though in fact, using this method it would be impossible to record a paternity share of 0 because at least one chick is needed with a female to count her as a genetic mating partner.

Standardised variance in mate number, paternity share, and mate fecundity were calculated in the same way: generating a single figure per male per week, and then averaging for each male across weeks before taking the variance across all males and dividing by the square of the mean.

This analysis was conducted using RStudio (RStudio_Team 2020).

Resampling technique to test for male phenotypic variance

Using the same data, we were able to estimate the proportion of I and I_s in single-sire flocks that may be associated with male identity, rather than being explained by variance between females. A resampling-based method was used (inspired by Fieberg, Vitense, and Johnson 2020), where the real mean weekly number of chicks over the first five weeks of hatching of the 144 females in each flock was "reshuffled" (resampled without replacement) among males.

Since the total population of 144 females is identical for each reshuffling, the mean chicks per male and per female will not vary between reshufflings. The variance among the population of females will also remain fixed. However, between-male variance in number of chicks and number of partners with whom chicks were produced will change with each reshuffle.

10,000 randomly reshuffled flocks were generated, and between-male I and I_s calculated for each one. The number of potential arrangements is extremely large (see footnote for calculation) [1], and so we can expect our sample of 10,000 combinations to be essentially free from repetition.

For each randomly "reshuffled" flock, between-male I was calculated. This process was repeated to generate 10,000 "reshuffled" values for each of the two single-sire flocks, which gave two

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[1] The equation $\frac{n!}{r!(n-r)!}$ gives the number of ways of selecting r elements without replacement from a list of n. In this case we are first selecting 12 elements from 144, then selecting 12 from (144 – 12), etc, which gives $\frac{(12 \times 12)!}{12!(11 \times 12)!} \times \frac{(11 \times 12)!}{12!(10 \times 12)!} \times \ldots \times \frac{(1 \times 12)!}{12!(0 \times 12)!}$. This simplifies down to $\frac{144!}{12 \times 12!}$. However, because the order of the groups does not matter, we also divide by 12!, which is the number of ways of permuting 12 groups. This gives us a final number of distinct reshufflings of $\frac{144!}{13!}$.

approximately Normally distributed sets of estimates for between-male I with females randomised between males. This analysis was carried out using self-written code in RStudio, mostly base R, rather than any specialised bootstrapping software (RStudio_Team 2020).

The logic of this is that we expect some of I and I_s to be caused by the fact that females will vary in the number of chicks they produce, independently of which male they are housed with. This variance can be explained as female phenotype plus residual random variance. By "reshuffling" females between males, we are determining a range of plausible values of I if the chick output of females is independent of which male they are housed with.

By contrast, if male phenotype also contributes to I, then the chick output of females would not be independent of which male they are housed with. We would expect females with high or low chick output to be clustered with particular males, because the phenotype of the male is influencing the number of chicks produced.

The most parsimonious assumption is that variation is random with respect to male phenotype. Therefore, we reject the null hypothesis that variation is not associated with male phenotype only if the observed value of I (or I_s) is greater than the estimates produced by reshuffling, i.e. if it is outside the 95% confidence interval given by the percentiles of the distribution of values of I generated from reshuffled females. The distance between the true value of I and the upper bound of this confidence interval represents the value of I that can be reasonably attributed to male phenotype. If, on the other hand, the true value of I is within the confidence interval produced by reshuffling females, we should retain the null hypothesis that I is entirely attributable to female phenotype and residual variance. One-sided p-values for the hypothesis that our observed values were generated by the same data generating process that generated the simulated distribution (i.e. no effect of male ID) were calculated as the number of simulated values that were equal to or above the observed value, divided by 10,000, the total number of simulated values (Ruxton and Neuhäuser 2013).

Behavioural Phenotype

Behavioural observations were carried out at farm A (single sire) in November 2019, and at farm B (bulk) in November 2021. Birds were in peak production, around 30 weeks old, at the time they were studied. The long gap between behavioural observations of the two farms was due to national coronavirus lockdowns and then restrictions put in place to limit the spread of avian influenza.

Each male was studied for a total of 180 minutes of focal watches, split over eight days in a fully time-balanced design. Four blocks of observation took place each day: between 08:00 and 09:30; between 09:30 and 11:00; between 12:00 and 13:30; and between 13:30 and 15:00. Each male had two 20-minute focal watch sessions during each of the four blocks, spread over eight separate days of observation.

During each focal watch, behaviours were recorded as described in ***Table 1.*** In some cases, different behavioural variables were recorded for bulk and single sire males. For males in single-sire flocks, it was usually possible to record the identity of females with whom males interacted; in bulk flocks, this was not possible. No male-male interactions could occur in single sire groups so these were not recorded. In addition, crows were only recorded for bulk males because I did not think of it when I observed the single sire males at farm A.

Table 1: *Ethogram used for collection and interpolation of behavioural data*

Behavioural measure	Explanation	Groups for which this variable was used
Total successful copulatios	Total number of successful copulations seen, where a successful copulation is determined by cloacal contact between male and female, either directly observed or inferred from tail position.	Bulk and single sire
Copulation attempt	Total number of copulation attempts seen, whether successful or unsuccessful. An attempt was counted if any of the following was observed: • Male approaches female from behind with hackles raised • Male grasps female's comb or head/neck feathers in his beak • Male places one foot on the back of a female • Male mounts the female with both feet on her back	Bulk and single sire
Success rate of copulation attempts	The proportion of attempts resulting in successful copulation. This was calculated by dividing the number of copulations by the number of attempts for each of the eight observations of each male, and then taking a mean across all observation sessions for each male	Bulk and single sire
Number of copulation partners	Number of unique partners a male was observed copulating with per observation session, averaged across sessions	Single sire only (unique IDs of females not known for bulk males)
Number of attempt partners	Number of unique partners with whom a male attempted copulation per observation session, averaged across sessions	Single sire only (unique IDs of females not known for bulk males)

Solicitations received	Number of times females crouch in front of males to invite copulation	Bulk and single sire
Courtship	An instance of courtship behaviour was counted if males made a food call (low clucking sound while scratching and pecking at the ground or picking up an item in his beak) or waltzed at a female (turned in an arc in front of her with outer wing outstretched and beating against leg)	Bulk and single sire
Crow	Distinctive loud call made by males with beak open and neck outstretched	Bulk only (did not collect for single sire)
Attack	All instances of aggression including pecking, chasing, or lunging at another male, waltzing at him (see Courtship), or interrupting his mating attempt.	Bulk only (no interaction between males occurs in single sire)
Waltz (between males)	As described in Courtship, but between two males instead of a male and a female. Record whether male performed or received waltz.	Bulk only (no interaction between males occurs in single sire)
Interrupt mating attempt	One male runs towards or attacks another while the latter is making a mating attempt. He may also try to physically displace the copulating male or insert himself between the copulating pair.	Bulk only (no interaction between males occurs in single sire)

To determine the relationship of each of the behavioural measures listed in ***Table 1*** with reproductive success and mating success, linear models were fit using each behavioural variable in turn as a predictor for reproductive success (mean-standardised weekly chicks) and mating success (mean-standardised genetic mating partners). Separate models were fit for bulk and single sire males, since it was plausible that the different dynamics of these two systems would lead to different behavioural variables modulating success. Each model specified different slopes and intercepts for the two twelve-male groups included. Mean-standardisation of mating and reproductive success was specific to the mating group (Bulk 1, Bulk 2, Single Sire 1, Single Sire 2).

To determine significance of behavioural variables as predictors of mating or reproductive success, each model was then compared to a null model in which group identity alone was a predictor (see ***Table 2***). Model comparison via likelihood ratio testing was used to ascertain whether each behavioural variable added statistically significant predictive power. The Benjamini-Hochberg procedure was used to correct for multiple comparisons with each of the four dependent variables (reproductive success and mating success for bulk and single sire) (Benjamini and Hochberg 1995).

Table 2: *Model comparison used to test significance of behavioural variables as determinants of fitness (either reproductive success, measured as weekly average chicks, or mating success, measured as weekly average genetic mating partners, both mean-standardised with respect to the mean value of the specific flock). Each model contained 24 data points representing the 24 total males observed in either the bulk or the single sire mating system. Each group consisted of 12 males; there were two groups studied in both mating systems.*

Null model	`Fitness ~ Behavioural variable + Group + Behavioural variable * Group`
Alternative model	`Fitness ~ Group`

A principal components analysis was carried out to test whether any correlated behavioural variables could predict mating success or reproductive success. However, the conclusions of this analysis were of little biological relevance. The methods and results of this further analysis are discussed in the appendix to this chapter.

Results

Total opportunity for selection in males

The total opportunity for selection in males is presented for all four groups in ***Figure 2.*** This is the standardised variance in male reproductive success (reproductive success measured as mean chicks hatched per week during the first five weeks of production) and encompasses all sources of variation, including male phenotype, female phenotype, male-female interaction effects, and residual random variation. For the two groups under the 'bulk' system, this will also encompass variation caused by intrasexual competition between males, whereas for the two groups under the 'single sire' mating system, there will be no variation caused by male intrasexual competition.

I was higher in both bulk mating groups (values 0.133, 0.288) compared to both single sire mating groups (0.024, 0.106. However, the differences between groups under the same mating system are striking: Bulk 2 has more than twice as much opportunity for selection as Bulk 1, while Single Sire 2 has more than four times as much opportunity for selection as Single Sire 1.

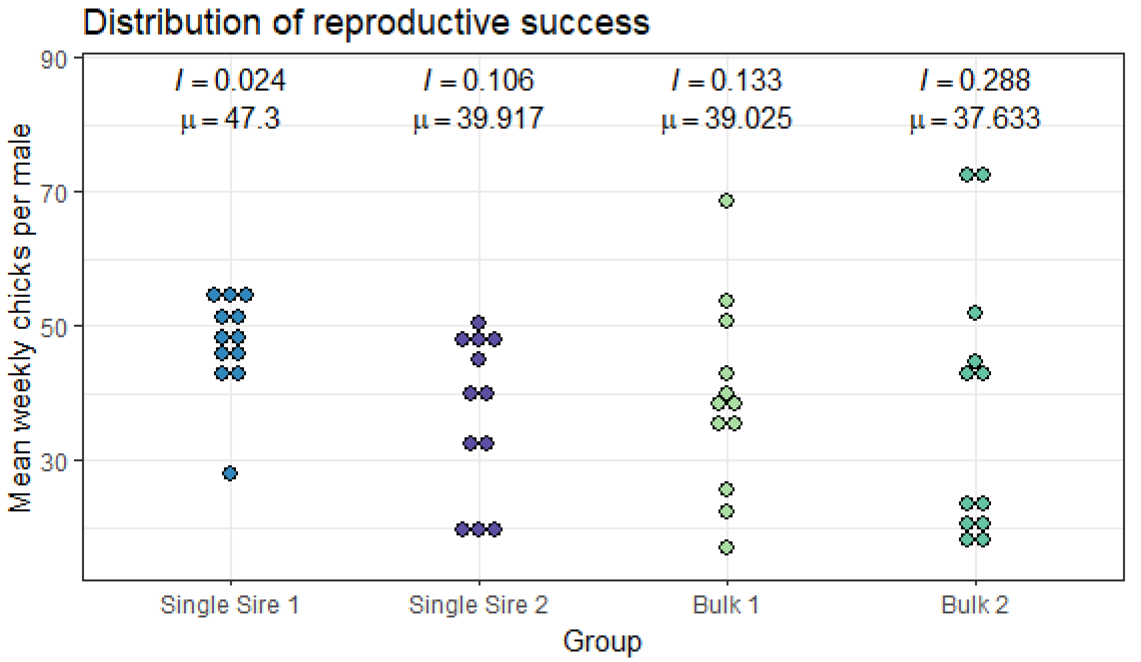

Figure 2: *Variance in reproductive success across the four groups studied. Each dot represents the reproductive success (mean weekly chicks hatched in the first five weeks of production) of one male. Single sire groups (no male intrasexual competition) are represented in blue, and bulk mating groups (male intrasexual competition possible) are represented in green. Standardised variances and means are given for each group at the top of the column.*

Opportunity for sexual selection in males

The difference in values of *I* between the two mating systems represents the opportunity for selection which may be attributable to male intrasexual competition, and therefore comes closer to the "true" opportunity for sexual selection in males (see ***Figure 4***). The smallest estimate is given by subtracting the largest single sire value from the smallest bulk value, and vice versa. This means that estimates of anywhere from 20% to 92% of total *I* being attributable to sexual selection are supported by this study. (***Table 3***)

Table 3: *Minimum and maximum estimates of total* I *attributable to intrasexual competition that are supported by this study. See* ***Figure 4*** *for visualisation of these estimates.*

	Minimum sexual selection	Maximum sexual selection
Total *I*	0.133 (Bulk Group 1)	0.288 (Bulk Group 2)
Estimate for *I* not attributable to sexual selection	0.106 (Single Sire Group 2)	0.024 (Single Sire Group 1)
Estimated opportunity for sexual selection (= bulk *I* minus single sire *I*)	0.027 (20% of total)	0.264 (92% of total)

Partitioning of non-competitive variance in reproductive success

In single-sire flocks, where there is no male intrasexual competition, some variance may be caused by non-competitive aspects of male phenotypes that influence mating and fertilising ability. Some may also be caused by differences in the females to which he is assigned, and random noise. We use a resampling-based method to estimate the size of this non-phenotypic *I* in single-sire flocks.

For both single-sire flocks, the observed value of *I* was outside the interval predicted without male phenotypic variance (Group 1: $p_{rand} = 0.002$; Group 2: $p_{rand} < 0.0001$; p values obtained by direct comparison with quantiles of null hypothesis distributions shown in ***Figure 3***). We can conclude that there is opportunity for selection due to male phenotype in both single sire groups. However, this opportunity varied substantially between the two groups studied, both in absolute magnitude and as a percentage of the total (see ***Table 4***).

Table 4: *Proportions of total* I *that may be associated with male identity in both single sire groups studied (with no intrasexual competition).*

	Minimum *I* attributed to male identity: Single Sire Group 1	Maximum *I* attributed to male identity: Single Sire Group 2
Total *I*	0.024	0.106
95% CI for *I* explained by non-phenotypic sources	0.003 – 0.016	0.009 – 0.050
I attributable to male phenotype (total minus upper bound of CI)	0.008 (24% of total)	0.056 (53% of total)

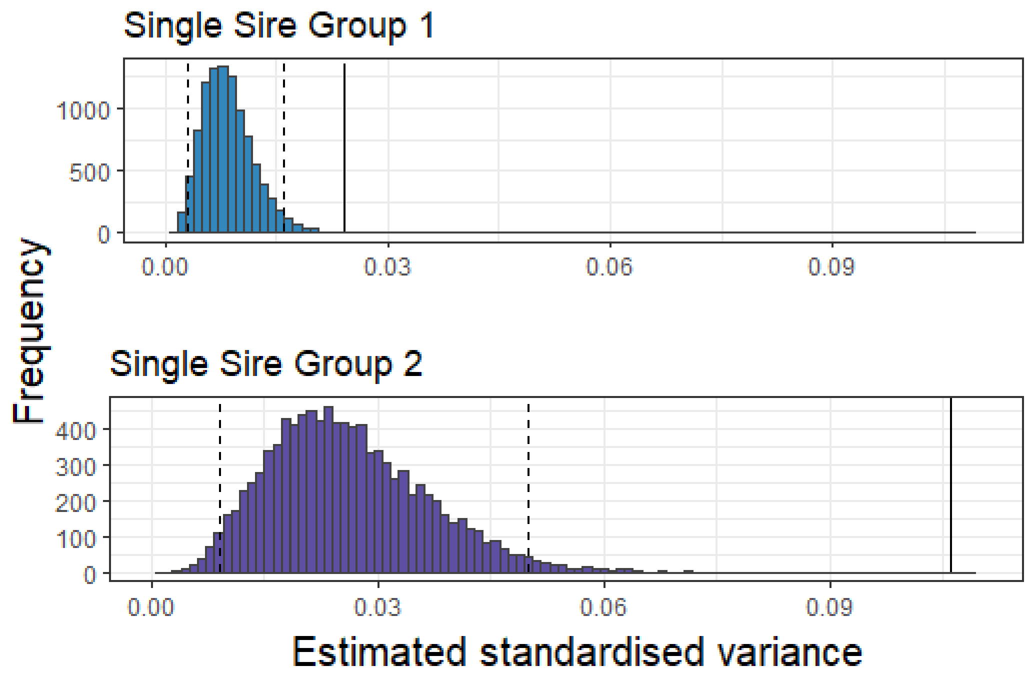

Figure 3: *Histograms showing range of estimates for between-male I attributable to female effects and stochastic variance in both single-sire flocks studied (see **Methods**). Dotted vertical lines show 95% confidence intervals for this distribution. Solid vertical lines show the observed values of I for each of these flocks. The difference between observed values and the upper bound of the distribution represent the opportunity for selection that is attributable to male phenotypic effects in the absence of male intrasexual competition, and having taken into account variation that may be attributable to other sources.*

Partitioning total opportunity for selection into different sources

The partitioning of total opportunity for selection in males is illustrated in ***Figure 4***. Estimates are given for the proportion of *I* that might be caused by intrasexual competition (ie the 'true' opportunity for sexual selection), that caused by differences in male phenotype outside of intrasexual competition, and residual variance explained by female phenotype and other factors.

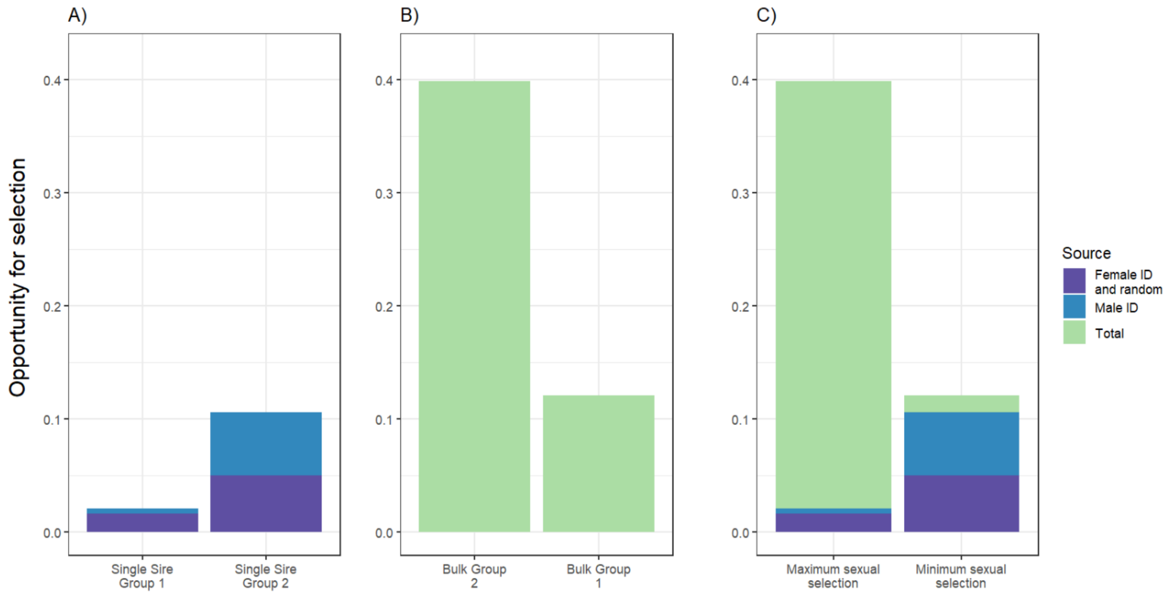

Figure 4: *Display of the range of estimates supported by this study for the true opportunity for sexual selection, and for the opportunity for non-competitive selection on male phenotype. Figure* ***A)*** *shows total values of I calculated for both single-sire groups (total height of bar), broken down into variance attributable to male phenotype (light blue) and variance attributable to other sources, including female phenotype and random error (dark blue) (see main text).* ***B)*** *shows total values of I calculated for both bulk-mating groups. In* ***C)*** *these are tentatively superimposed, showing the maximum and minimum difference between* ***A)*** *and* ***B)****. The difference between total I in bulk mating and single sire flocks (visible portion of light green bars) may be attributable to intrasexual competition and thus could represent the 'true' opportunity for sexual selection.* ***C)*** *should be interpreted with caution, because there is no guarantee that the magnitude of non-competitive opportunity for selection would be the same in the two bulk mating flocks as it was in the single-sire flocks studied.*

Variance in male mating success

Variance between males in genetic mating success for all four groups in ***Figure 5***. The standardised variance is greater in 'bulk mating' groups than 'single sire'; visually, we can see that variation in 'single sire' groups is curtailed by the upper limit of twelve females available to each male. By contrast, genetic mating success for males in 'bulk' groups does not come near to its upper limit of 144, though the mean number of genetic partners per male is roughly twice as high for bulk males as single sire males. Although I_s is typically used as a measure of opportunity for sexual selection, we can see that it is above zero in both 'single sire' groups, where the *true* opportunity for sexual selection is zero, since there is no opportunity for male intrasexual competition to take place.

As expected, however, I_s is higher in both bulk mating groups than in both single sire groups (0.102 and 0.172 vs 0.020 and 0.067), as with total opportunity for selection. As with total opportunity for selection, variability between groups under the same mating system is striking. In particular, I_s is over three times higher in single sire group 2 than in single sire group 1.

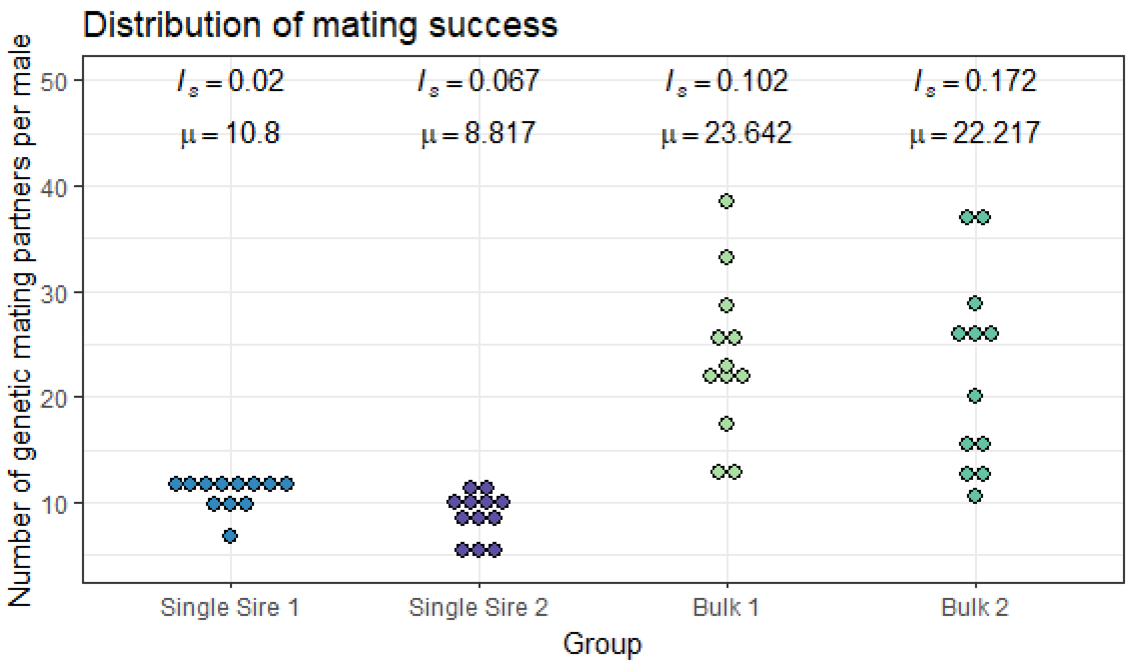

Figure 5: *Variance in mating success across the four groups studied. Each male's mating success is measured as the mean number of genetic mating partners per week across the first five weeks of production. Each dot represents the performance of one male. Single sire groups are represented in blue, and 'bulk mating' groups in green. For 'single sire' groups, the potential number of mating partners for males is capped at 12, and for 'bulk mating' males the limit is 144. Standardised variances and means are given for each group at the tops of columns.*

This variability produces a wide range for the estimated proportion of I_s that is attributable to male intrasexual competition. This estimate is derived from the difference in I_s between bulk and single sire flocks, and represents the variance in mating success upon which sexual selection may act (see ***Table 5*** and ***Figure 7***).

Table 5: *Calculation of the minimum and maximum opportunity for sexual selection on mating success. The minimum estimate comes from subtracting the largest single-sire* I_s *from the smallest bulk* I_s*, and vice versa for the maximum estimate.*

	Minimum opportunity for sexual selection on mating success	Maximum opportunity for sexual selection on mating success
Total I_s	0.102 (Bulk Group 1)	0.172 (Bulk Group 2)
Estimate for I_s not attributable to sexual selection	0.067 (Single Sire Group 2)	0.020 (Single Sire Group 1)
Estimate for I_s attributable to sexual selection (= bulk I_s minus single sire I_s)	0.035 (34% of total)	0.152 (88% of total)

Non competitive male phenotypic variance in I_s

The same "reshuffling" procedure was used to determine the extent to which I_s in single-sire flocks can be attributed to male identity (***Figure 5***). In both single-sire flocks, observed values of I_s were well above predicted values if male phenotype did not influence mating success (Single Sire Group 1: $p < 0.0001$; Single Sire Group 2: $p < 0.0001$; p values obtained by direct comparison with quantiles of null hypothesis distribution). We can conclude, therefore, that there is opportunity for selection on mating success associated with male identity in single sire flocks (ie, where there is no intrasexual competition between males).

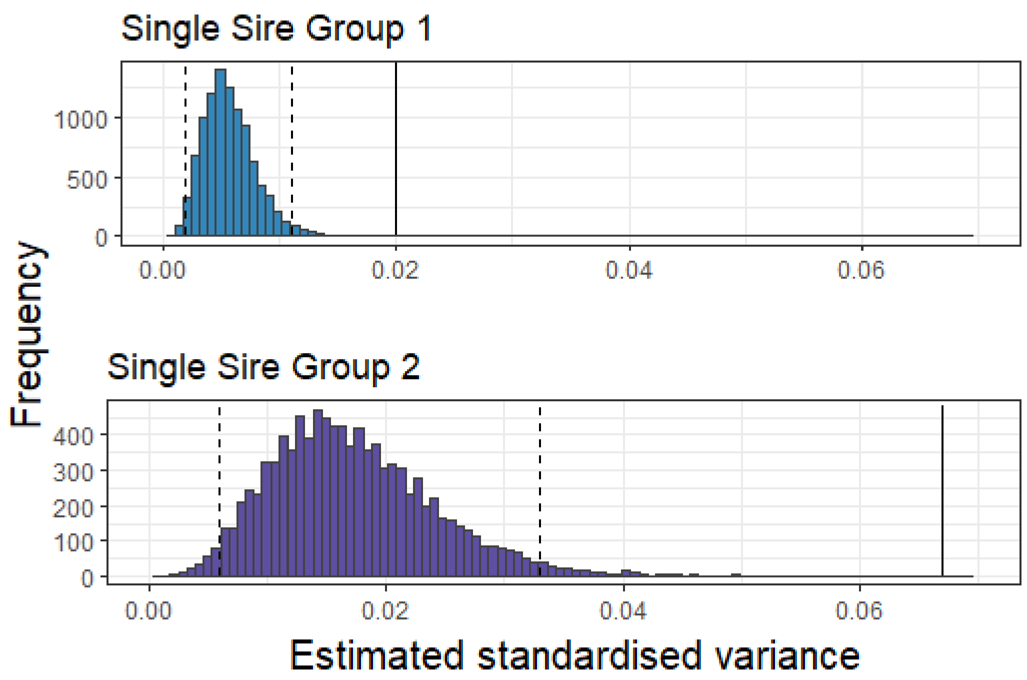

Figure 6: *Histograms showing range of estimates for between-male I_s attributable to sources other than male identity in both single-sire flocks studied (see* ***Methods****). Dotted vertical lines show 95%*

confidence intervals for this distribution. Solid vertical lines show the observed values of $\mathrm{I_s}$ *for each of these flocks. The difference between observed values and the upper bound of the distribution represent the value of* $\mathrm{I_s}$ *that is attributable to male identity in the absence of male intrasexual competition.*

Table 6: *Proportions of total* $\mathrm{I_s}$ *that may be associated with male identity in both single sire groups studied (with no intrasexual competition).*

	Minimum I_s attributed to male identity: Single Sire Group 1	Maximum I_s attributed to male identity: Single Sire Group 2
Total I_s	0.020	0.067
95% CI for I_s independent of male identity	0.002 – 0.011	0.006 – 0.033
I attributable to male identity (total minus upper bound of CI)	0.009 (45% of total)	0.034 (51% of total)

Partitioning I_s into different sources

As with variance in reproductive success, variance in mating success can be compared for single-sire and bulk mating groups to provide an estimate for the variance that is attributable to intrasexual competition. Of variance which is not attributable to intrasexual competition (because it occurred in a single-sire group), we can calculate the portion that is attributable to male identity (and therefore upon which selection might act) and the portion that is independent of male identity. This is illustrated in ***Figure 7***.

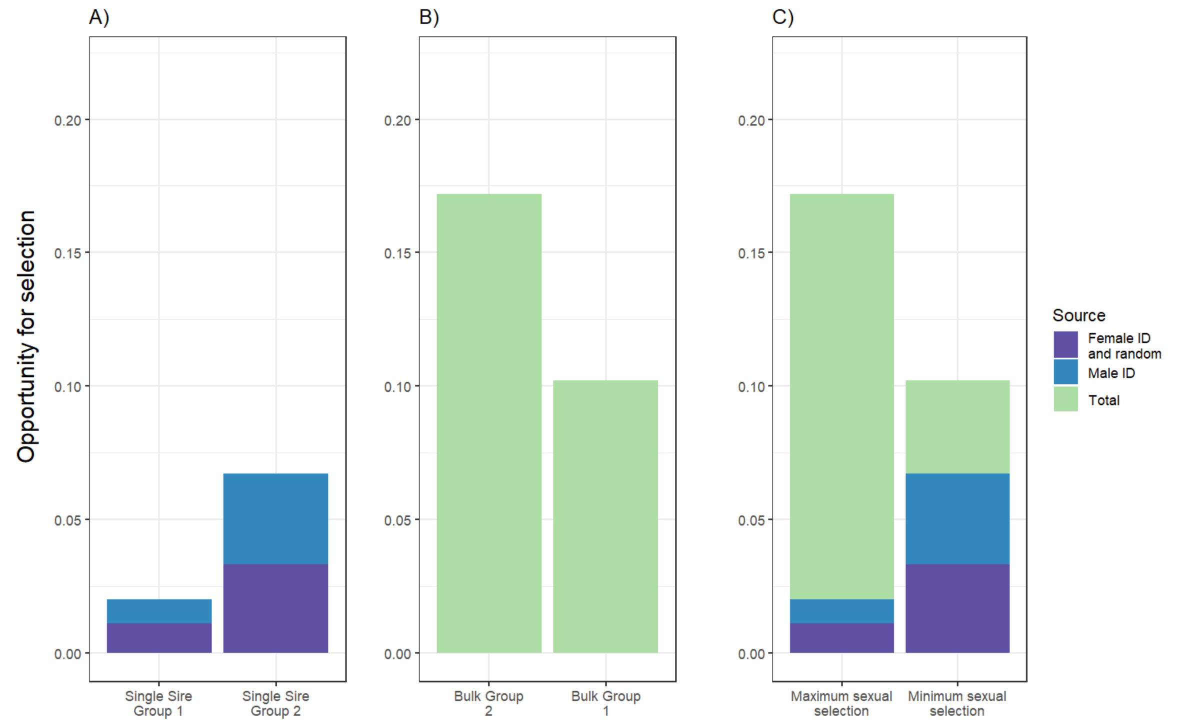

Figure 7: *Display of the range of estimates supported by this study for the true opportunity for sexual selection via mating success, and for the size of* I_s *associated with male identity. Figure* ***A)*** *shows total values of* I_s *calculated for both single-sire groups (total height of bar), broken down into variance attributable to male identity (light blue) and the upper bound of the variance attributable to other sources, including female phenotype and random error (dark blue) (see main text).* ***B)*** *shows total values of* I_s *calculated for both bulk-mating groups. In* ***C)*** *these are tentatively superimposed. The difference between total* I_s *in bulk mating and single sire flocks (visible portion of light green bars) may be attributable to intrasexual competition and thus represents the 'true' opportunity for sexual selection on mating success.*

Paternity share

Variance in paternity share across all groups is shown in ***Figure 8***. A male's paternity share is calculated as the mean proportion of chicks sired across all of his genetic mating partners, per week, averaged over the first five weeks of production. The standardised variance in this measure is zero for both single sire groups, because in this mating system every male sires all the chicks of each of his genetic mating partners. The paternity share of males in this system will always be 1. By contrast, we find that in the bulk mating groups, males sire on average just over a third of the chicks of each of their genetic mating partners, and that there is some variation between males.

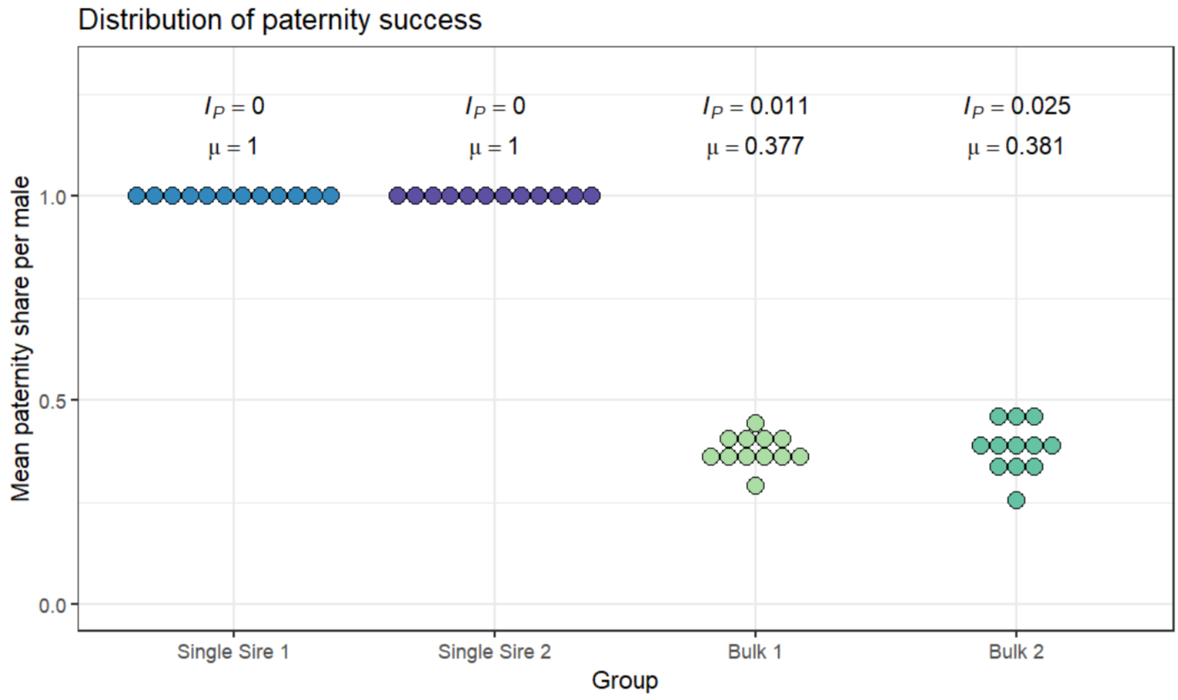

Figure 8: *Variance in paternity share across the four groups studied. Single sire groups are represented in blue, and bulk mating groups in green. Standardised variances and means are given for each group at the tops of columns.*

Variance in mate fecundity

Variance in mate fecundity across each of the four groups is shown in ***Figure 9***. A male's mate fecundity is calculated as the mean number of chicks produced week by all his genetic mating partners in a week, averaged across the first five weeks of production. Variance in mate fecundity is extremely small, and is not increased in bulk groups compared to single sire groups, suggesting that sexual selection does not on males act via competition for high fecundity mates.

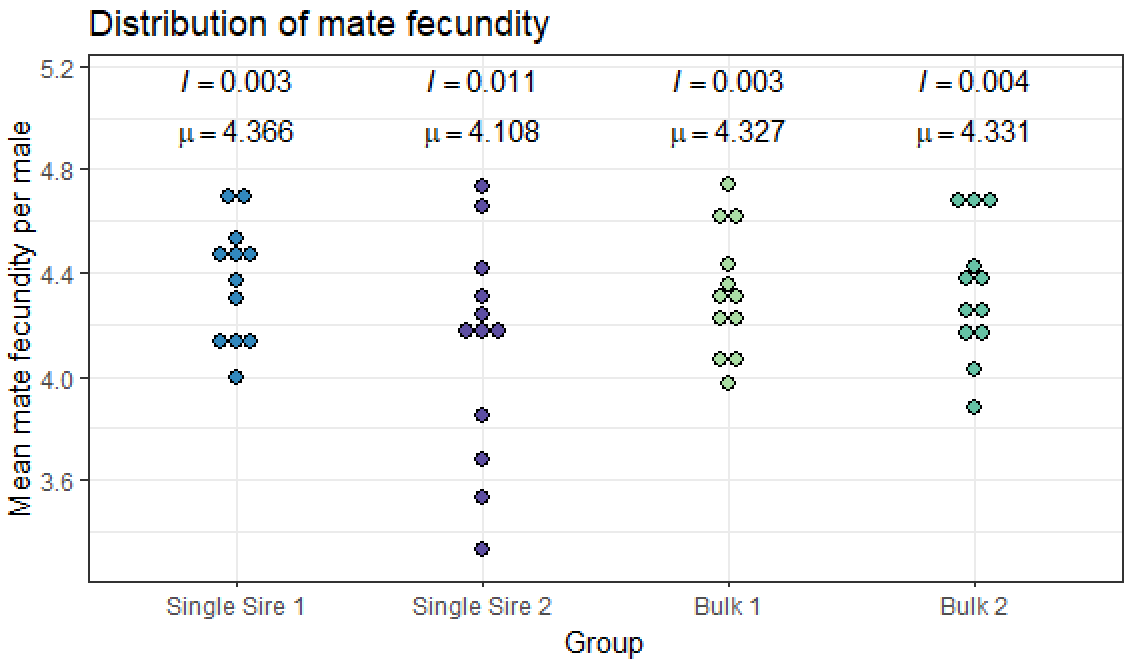

Figure 9: *Variance in mate fecundity across the four groups studies. Single sire groups are represented in blue, and 'bulk mating' groups in green. Standardised variances and means are given for each group at the tops of columns.*

Decomposition of total reproductive success

These three components of variance, as well as the total variance, are plotted alongside each other for the four flocks studied in ***Figure 10***. We can see that of the three components, mating success consistently contributes by far the largest amount of variance across each of the four flocks. We can also see that there is a surprising level of variation between flocks of the same mating system in the magnitudes of variance that they show. Single Sire 2 and Bulk 1 both look more similar to each other than they do to other flocks of the same mating types, which we would expect them to resemble. As expected, variance in each of the components add up to close to the total variance in both single sire flocks and in Bulk 1 (Collet et al. 2012). However, Bulk 2 has a large amount of residual variance in reproductive success that does not seem to be explained by variance in any of mating success, paternity share or partner fecundity.

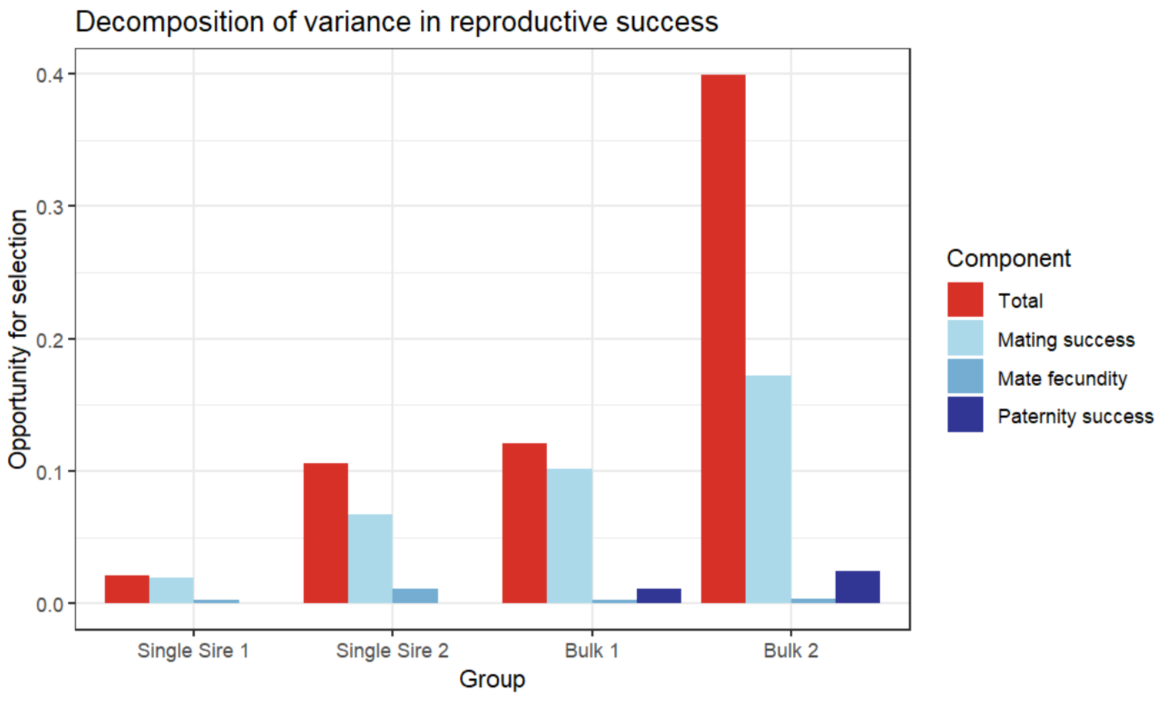

Figure 10: *Bar plot showing the total opportunity for selection for each group studied compared to the size of different multiplicative components: mating success, mate fecundity, and paternity success.*

Behavioural Phenotype

Results of likelihood ratio tests between null and alternative models to test significance of associations between behavioural variables and fitness are given in ***Table 7***. No statistically significant effects were detected.

Table 7: *Test statistics for relationships between individual-level behavioural measures and reproductive or mating success*

Behavioural measure	Reproductive success (mean-standardised chicks)		Mating success (mean-standardised genetic partners)	
	Bulk	Single Sire	Bulk	Single Sire
Copulations	χ^2 = 1.398, df = 2, $p_{unadjusted}$ = 0.497, $p_{adjusted}$ = 0.773	χ^2 = 5.636, df = 2, $p_{unadjusted}$ = 0.060, $p_{adjusted}$ = 0.231	χ^2 = 2.211, df = 2, $p_{unadjusted}$ = 0.331, $p_{adjusted}$ = 0.756	χ^2 = 4.547, df = 2, $p_{unadjusted}$ = 0.103, $p_{adjusted}$ = 0.361

Copulation partners observed	n/a	$\chi^2 = 3.641$, df = 2, $p_{unadjusted} = 0.162$, $p_{adjusted} = 0.284$	n/a	$\chi^2 = 2.762$, df = 2, $p_{unadjusted} = 0.251$, $p_{adjusted} = 0.454$
Copulation attempts	$\chi^2 = 2.204$, df = 2, $p_{unadjusted} = 0.332$, $p_{adjusted} = 0.694$	$\chi^2 = 0.853$, df = 2, $p_{unadjusted} = 0.653$, $p_{adjusted} = 0.653$	$\chi^2 = 2.678$, df = 2, $p_{unadjusted} = 0.262$, $p_{adjusted} = 0.756$	$\chi^2 = 0.954$, df = 2, $p_{unadjusted} = 0.621$, $p_{adjusted} = 0.701$
Attempt partners observed	n/a	$\chi^2 = 2.201$, df = 2, $p_{unadjusted} = 0.333$, $p_{adjusted} = 0.466$	n/a	$\chi^2 = 2.414$, df = 2, $p_{unadjusted} = 0.299$, $p_{adjusted} = 0.454$
Copulation success (successful/all attempts)	$\chi^2 = 2.119$, df = 2, $p_{unadjusted} = 0.347$, $p_{adjusted} = 0.694$	$\chi^2 = 4.480$, df = 2, $p_{unadjusted} = 0.107$, $p_{adjusted} = 0.250$	$\chi^2 = 2.433$, df = 2, $p_{unadjusted} = 0.296$, $p_{adjusted} = 0.756$	$\chi^2 = 2.253$, df = 2, $p_{unadjusted} = 0.324$, $p_{adjusted} = 0.454$
Solicitations received	$\chi^2 = 2.990$, df = 2, $p_{unadjusted} = 0.224$, $p_{adjusted} = 0.694$	$\chi^2 = 1.031$, df = 2, $p_{unadjusted} = 0.507$, $p_{adjusted} = 0.592$	$\chi^2 = 3.255$, df = 2, $p_{unadjusted} = 0.196$, $p_{adjusted} = 0.756$	$\chi^2 = 0.712$, df = 2, $p_{unadjusted} = 0.701$, $p_{adjusted} = 0.701$
Courtship (food calls and waltzes)	$\chi^2 = 5.370$, df = 2, $p_{unadjusted} = 0.068$, $p_{adjusted} = 0.511$	$\chi^2 = 5.447$, df = 2, $p_{unadjusted} = 0.066$, $p_{adjusted} = 0.231$	$\chi^2 = 5.028$, df = 2, $p_{unadjusted} = 0.081$, $p_{adjusted} = 0.581$	$\chi^2 = 5.908$, df = 2, $p_{unadjusted} = 0.052$, $p_{adjusted} = 0.361$
Crows	$\chi^2 = 5.236$, df = 2, $p_{unadjusted} = 0.073$, $p_{adjusted} = 0.511$	n/a	$\chi^2 = 4.976$, df = 2, $p_{unadjusted} = 0.083$, $p_{adjusted} = 0.581$	n/a
Aggression given	$\chi^2 = 1.750$, df = 2, $p_{unadjusted} = 0.417$, $p_{adjusted} = 0.730$	n/a	$\chi^2 = 1.263$, df = 2, $p_{unadjusted} = 0.532$, $p_{adjusted} = 0.828$	n/a
Aggression received	$\chi^2 = 1.007$, df = 2, $p_{unadjusted} = 0.604$, $p_{adjusted} = 0.846$	n/a	$\chi^2 = 0.763$, df = 2, $p_{unadjusted} = 0.683$, $p_{adjusted} = 0.874$	n/a
Times interrupted	$\chi^2 = 0.252$, df = 2, $p_{unadjusted} = 0.882$, $p_{adjusted} = 0.923$	n/a	$\chi^2 = 0.525$, df = 2, $p_{unadjusted} = 0.769$, $p_{adjusted} = 0.897$	n/a
Times interrupted other males	$\chi^2 = 0.262$, df = 2, $p_{unadjusted} = 0.877$, $p_{adjusted} = 0.923$	n/a	$\chi^2 = 0.752$, df = 2, $p_{unadjusted} = 0.687$, $p_{adjusted} = 0.874$	n/a
Waltzes to other males	$\chi^2 = 2.171$, df = 2, $p_{unadjusted} = 0.338$, $p_{adjusted} = 0.694$	n/a	$\chi^2 =1.568$, df = 2, $p_{unadjusted} = 0.457$, $p_{adjusted} = 0.800$	n/a

Waltzes from other males	$\chi^2 = 0.161$, df = 2, $p_{unadjusted} = 0.923$, $p_{adjusted} = 0.923$	n/a	$\chi^2 = 0.068$, df = 2, $p_{unadjusted} = 0.967$, $p_{adjusted} = 0.967$	n/a
Total aggression (incl waltzes and interruptions)	$\chi^2 = 2.620$, df = 2, $p_{unadjusted} = 0.270$, $p_{adjusted} = 0.694$	n/a	$\chi^2 = 1.945$, df = 2, $p_{unadjusted} = 0.378$, $p_{adjusted} = 0.756$	n/a
Total aggression received	$\chi^2 = 0.405$, df = 2, $p_{unadjusted} = 0.817$, $p_{adjusted} = 0.923$	n/a	$\chi^2 = 0.161$, df = 2, $p_{unadjusted} = 0.923$, $p_{adjusted} = 0.967$	n/a

Results of principal components analysis are given in the appendix to this chapter.

Discussion

As expected, our results are in accordance with the idea that opportunity for selection in males is increased by intrasexual competition. Our observed values of I in bulk groups were 0.133 and 0.288, which range from 1.2 times to 12 times the values observed in single sire groups, where intrasexual competition was absent. By far the largest identifiable component was variance in mating success, I_s, which was larger in bulk groups than single sire. Variance in paternity share, which in single sire groups is zero, saw only a small increase in bulk groups. Variance in partner fecundity was the smallest identifiable component in bulk groups, and was not larger in bulk groups than in single sire.

Compared to a previous experiment that estimates I, I_s, and variance in paternity share and in mate fecundity in red junglefowl males, our observed values are fairly small (McDonald et al. 2017). Factors which may contribute to explaining this discrepancy include strong artificial selection reducing variance in broiler breeders, and the fact that reliance on genetic mating data means some values are systematically missing from our dataset, decreasing variance. Both of these are discussed below. In addition, the red junglefowl estimates are based on groups containing males of a range of ages, whereas in this study all birds within each flock were the same age.

Our results demonstrate the multiple problems inherent in the use of I_s, or standardised variance in mating success, as a measure of the opportunity for sexual selection. Firstly, mating success (M) is just one component of male reproductive success. Male reproductive success is also determined by variance in paternity share (P) and in mate fecundity (N) (***Figure 1*** in **Introduction**). If intrasexual competition between males contributes to variance in either of these components, then this is opportunity for sexual selection that is not captured by I_s (error **a)** as set out in the **Introduction**). We observe an increase in variance in P in bulk groups compared to single-sire groups, where all males have 100% paternity share and variance in P is zero. The variance in P in bulk groups therefore represents an opportunity for sexual selection that does not contribute to I_s. The magnitude of this source of error seems to be relatively small in this system, however, because standardised variance in P was small (0.014 and 0.019, roughly a tenth of the magnitude of observed values of I_s of 0.102 and 0.172).

Error is also introduced by the fact that not all of I_s is attributable to intrasexual competition (error **b)** as outlined in the **Introduction**). Since sexual selection is defined as selection occurring due to intrasexual competition for mating opportunities, I_s in single-sire flocks cannot properly be considered as opportunity for sexual selection, because no intrasexual competition takes place in this mating system. Opportunity for sexual selection ought to represent the largest possible selection differential for a perfectly heritable trait that was *only* sexually selected, which in these populations would be zero. We observe values of I_s in single-sire flocks of that are from 12% to 66% of the values observed in bulk flocks. This implies that up to two thirds of “opportunity for sexual selection” may not truly be opportunity for sexual selection, which is concerning because this measure is frequently used without such caveats (H Klug et al. 2010).

In our analysis, we considered the “true” opportunity for sexual selection to be the difference between total opportunity for selection in bulk flocks, where sexual selection can act, and in single sire flocks, where it cannot. I in single sire flocks is taken to be an indication of the magnitude of I that may be attributable to sources other than intrasexual competition. Since we only have two estimates for I in each mating system, it is difficult to accurately estimate this value. In addition, there is no guarantee that I caused by non-competitive sources in single sire flocks provides an accurate indication of the same quantity in bulk flocks. However, we tentatively calculate our estimates for this value as a minimum of 0.027 and a maximum of 0.264, ranging from 20% to 92% of the total opportunity for selection in bulk mating groups. This is a notably wider range than our two estimates of I_s (0.102 and 0.172), a standard measure of the opportunity for sexual selection used by many authors (H Klug et al. 2010). This discrepancy may be due to error **a)** as discussed in the **Introduction**. I_s only takes into account a single component of reproductive success, and as such there is less possibility for it to vary between groups than I, which encompasses all components of reproductive success. On the other hand, however, components other than M were found to make a relatively small contribution to variance in reproductive success, which suggests that this cannot be a complete explanation.

Theory predicts that one driver of increasing I in the bulk groups is increased variance in mating success (also referred to as I_s). Our results confirm this: I_s was 1.5 times to 8.6 times larger in bulk groups than single sire groups. Previous work in this species has suggested that intrasexual competition between males means that low-ranking males suffer almost complete exclusion from mating opportunities (Guhl, Collias, and Allee 1945). However, this does not seem to have been the case in these flocks: male mating success is almost uniformly increased on an individual basis in bulk flocks compared to single sire flocks, with almost no overlap in the distribution of mating success between bulk and single sire flocks (see ***Figure 4***). This suggests that the increased variance in mating success in bulk flocks is driven more by the removal of the ceiling of twelve potential mating partners imposed in single sire flocks, and that monopolisation of females by dominant males is low. Higher-performing males do not seem to be obtaining more partners at the expense of lower-performing males; instead, increasing polyandry allows all males to either increase their mating success or at worst to remain level. By contrast, the variance increase in total reproductive success in bulk flocks compared to single sire is driven by an expanding range in both directions: the lowest performing males have fewer chicks, as well as the highest performing males having more. This can be explained by the change in average paternity share: if males go from a paternity share of 1 to around one third, then attaining the same number of mating partners will give you only one third as many chicks.

In single sire flocks, there is an artificial limit on mating success: a maximum of 12 partners are available. In bulk flocks, some other kind of limit on mating success seems to be in place, since the highest performing males have below 40 genetic mating partners in the average week: less than a third of the females theoretically available to them. This could be a result of intrasexual competition: although males don't seem to *completely exclude* competitors from all mating opportunities, it may be that some degree of competition prevents males from mating with every available female. Alternatively, there may be a constraint intrinsic to individual males: for instance, sperm limitation could prevent him from fertilising more females, or he could lack the motivation for frequent copulations. We could test for this by removing the ceiling imposed by the single sire competition but retaining the lack of intrasexual competition, and place one male in a pen with 144 females. If the mating success of males in this situation was higher than in our bulk flocks, then we could attribute the difference to the effects of intrasexual competition.

Although smaller than in bulk flocks, I_s in single sire flocks was surprisingly large: up to two thirds the value in bulk flocks. This is surprising since, in the absence of competition, there is "nothing stopping" males from mating with all available females. They cannot be aggressively excluded by competitors; female choice is likely to be minimal since females have no other potential mates available for the entirety of their reproductive lives, and are unlikely to choose to simply not mate. Because we are using genetic parentage data, mating success of less than 12 can have two possible causes: either males are not mating with all females, or, they are mating with all females but these pairings do not result in any chicks, even in the absence of competition. Both males and females can contribute to these two modes of failure (Assersohn, Brekke, and Hemmings 2021).

Our null hypothesis from the point of view of studying males should be that all variation is attributable to a combination of female factors and stochasticity. However, our determination of 95% confidence intervals for the variance which can be explained by factors other than male identity finds that approximately half the variance in mating success in each single sire flock is attributable to male identity; i.e., that some males have lower mating success than others and this is not simply due to bad luck, including the luck of which females he was assigned. There are several possible mechanisms that could cause this. For instance, some males might copulate less than others (making it more likely that not all available females will be genetic mating partners), whether because they show less motivation or because they are less attractive to females, even in the absence of any other males. Males might all copulate the same amount, but some might show greater choosiness, and neglect mating opportunities with some females. Alternatively, all males might copulate fully with all females, but some may be more likely to show fertilisation or hatching failure than others.

Theory also predicts that *I* should be increased in bulk flocks compared to single sire due to the potential for polyandry causing increased variance in paternity share (Geoff A Parker and Pizzari 2010). There is no variance in paternity share in the single sire flocks: all females have access to only one male, and so that one male fertilises one hundred percent of any chicks they produce. In a sense, therefore, all opportunity for selection due to variance in paternity share could be considered to have been caused by intrasexual competition and therefore to be part of sexual selection.

Our analysis finds that variance in paternity share is surprisingly small in comparison to total *I*, however. This is surprising as other studies suggest males vary substantially in ejaculate competitiveness (B Bilcik, Estevez, and Russek-Cohen 2005; Bowling et al. 2003; McDonald et al. 2017; Robertson et al. 1998). In addition, the reported variance in P is a lower proportion of total variance than has previously been reported in junglefowl (McDonald et al. 2017). However, our reliance on genetic mating data is likely to both overestimate the mean P and underestimate variance in P. Any males with a paternity share of zero with a given female (they mated but he did not sire any chicks) would not show up in our data; it is possible that this is common enough to create a significant distortion. Another way of looking at this is that, since females produced on average slightly over four chicks per week, the minimum detectable paternity share is around 25%.

Variance in P may also be underestimated by the fact that females lay a small, discrete number of hatching eggs per week. This means that the values of P a male can have with a particular female are constrained to be a discrete multiple of one quarter (or of one third, or one fifth, etc, depending on how many chicks a female produced that week). To get each male's value of P, this is then averaged across his genetic partners, of which he has on average approximately 23. This process seems mathematically inclined to produce a smaller variance across males compared to variance in mating success, where M can take any discrete value between 0 and 144 and does not undergo any kind of averaging. Arguably, a male's *true* value of P should be the *probability* that his sperm will fertilise an egg (which could take any continuous value between 0 and 1), not the outcome of this process, which for each egg becomes a binary of success versus failure.

Opportunity for selection due to mate fecundity was extremely low in all groups, and was not larger in bulk groups than single sire (0.003 in Bulk 1 and 2, and in Single Sire 1; 0.011 in Single Sire 2). The lack of increase observed in bulk flocks compared to single sire implies that there are not significant levels of competition between males over more fecund mates, and perhaps that females do not vary greatly in fecundity. This is not very surprising, because these birds have been under intense artificial selection for high fecundity, and in addition have been individually chosen to be part of the pedigree breeding group on account of their desirable attributes. This suggests that females may be close to their physiological maximum for fecundity, with little room for variability.

On the other hand, the fact that our calculation is based on genetic parentage data means that there may be additional females who are invisible to us: if a female mates with a male but does not produce any chicks with him, she will not contribute to his average partner fecundity. This is more likely to occur in females with lower fecundity, since a male has fewer chances to show up in the dataset as one of her genetic partners. This means that as well as being biased against males with low paternity success, our dataset is biased against females with low fecundity, and variance in partner fecundity may be underestimated. However, variance in this component is still very small compared to total variance or to variance in mating success, suggesting that there is very little opportunity for males to be selected for the ability to choose fecund mates.

One surprising result of this analysis is the variability between flocks. Both I and I_s were several times larger in single sire 2 than single sire 1, and in bulk 2 than in bulk 1. This is surprising because all birds were of the same pedigree line, and the husbandry setup between flocks of the same breeding system was held entirely constant: birds of the same breeding system were living inside the same house on the same farm at the same time. This may be an indication that inter-individual variability has a large influence on the opportunity for selection in groups when the environment is held constant. A reason for this could be that intense selection has pushed trait values towards their physiological limits, reducing variability within populations and increasing the effects of stochastic inter-individual variation on group measures. Since there is “no room” to *increase* reproductive success, we would expect occasional poor-performing individuals to have an outsize effect on increasing the variance of the group.

This is supported by the observation that single sire flock 1 has consistently both higher means and lower standardised variances than single sire flock 2 across reproductive success, mating success, and mate fecundity. This suggests that the single-sire females are operating close to a physiological ceiling of reproductive output. Observed values of I were low compared to McDonald et al.'s (2017) study of red junglefowl, again suggesting reduced variability as a result of either artificial selection or a highly uniform environment.

The opportunity for selection in commercial flocks may be reduced compared to free-living flocks, since strong artificial selection for commercially relevant traits is expected to reduce the phenotypic variance in the population. We might expect a greater proportion of variance to be attributable to stochasticity. In addition, we might expect a greater proportion to be attributable to social, rather than physical, traits. Social rank is a relative trait: to be dominant, an individual needs to be dominant *over someone else*. Although social dominance is somewhat heritable (Craig, Ortman, and Guhl 1965), it is impossible for the population average rank to increase, even if the population average of traits that contribute to rank (such as size and aggression) were to increase under selection (A. J. Moore et al. 2002). Therefore, we expect variance in contributory traits to decrease as they tend towards a natural physical limit, but for variance in social dominance to be maintained.

Another surprising result is the large variability in the amount of residual variance. The ratio of variance in T, M, N, and P remains roughly consistent across all four flocks studied, except for bulk flock 2, which has an observed variance in T almost twice as high as the sum of variances of M, N and P. The other three flocks conform to the expectation that variance in T should be roughly equal to the sum of the variance of each component (and their covariance) (Collet et al. 2012).

In single sire flocks, we expect that some variance in mating success and reproductive success is attributable to the phenotypes of males, and some is attributable to other sources such as the phenotypes of females in his pen. Since females are assigned to the male rather than in any way chosen by him, effects of their phenotypes can be considered stochastic variation from the point of view of the male. The null hypothesis is that all variance is random with respect to male phenotype. However, we determine that some portion of variance in both total reproductive success and mating success is attributable to male identity in both single sire flocks, as determined by subtracting the upper limit for the amount plausibly attributable to stochastic variation between females from the observed values (**Reproductive success:** Single Sire 1, 0.008; Single Sire 2, 0.056; **Mating success:** Single Sire 1, 0.009; Single Sire 2, 0.034). Although these are small opportunities for selection in absolute terms (especially for Single Sire 1), they are an appreciable fraction of the total variance in reproductive success or mating success observed (24%, 53%, 45%, and 51% respectively).

We do not know for certain that it is the phenotype of males that causes this variation: an alternative explanation would be that particular *pens* cause higher or lower success. We have established that being placed in a particular pen with a particular male influences a female’s chick output and the likelihood of being a genetic mating partner of the male she is housed with. Since the pens are almost entirely uniform and have much less potential to vary than males do, it seems very unlikely that they would be responsible for much of the variance. However, to rule out this possibility, we could conduct an experiment where males rotate around pens of females. If the pen itself is responsible for variance, we would expect female fertility to be unchanged; if males are responsible, we would expect patterns of high and low fertility to follow certain males as they moved between pens.

Determining that the identity of males is associated with variance in mating success or reproductive success strongly implies that some phenotypic feature or features causes greater or lesser success in some males. Notably, this is in the absence of intrasexual competition- so this trait or traits affect male success intrinsically, rather than just via an impact on competition with other males. It is highly likely that male identity *also* affects success via competition, but what we have done is show that male identity has an effect isolated from the effects of competition. However, this does not indicate *which* phenotypic traits of males may be associated with variance. In addition, it does not necessarily mean that this the traits responsible are under selection, because we do not know how heritable they are (Moiron, Charmantier, and Bouwhuis 2022).

In an attempt to identify traits associated with success in males, we investigated the behavioural phenotype of males, recording traits such as the number of copulations, the number of courtship behaviours, and the number of aggressive encounters initiated and received, during a total of 160 minutes per male of focal watching. However, we did not detect an association between any behavioural trait and either mating success or reproductive success in single sire or bulk males.

This may seem surprising, because both reproductive success and mating success might naively be expected to show a strong relationship with copulation frequency, and similarly with other behaviours that are part of the mating sequence. However, other researchers have also found a lack of relationship, or a weak relationship only, between copulation frequency and reproductive success in broiler breeder males (B Bilcik, Estevez, and Russek-Cohen 2005; I. J.H. Duncan, Hocking, and Seawright 1990; Paul M Hocking 2008)

There are two possible explanations for this null result. The first is that behavioural traits are not significantly associated with mating success or reproductive success in this situation. The second is that our length of observation was insufficient to provide an accurate picture of behavioural variation between males. In a principal components analysis, we find that the largest component loads positively on every behavioural trait, for instance both on initiating and on receiving aggression (see Appendix to this chapter). This might suggest that, due to low levels of general activity in broiler breeders (M S Dawkins and Layton 2012), behavioural variation between individual males can largely be summed up by the extent to which they show any activity at all. This might make relevant behavioural phenotypes more difficult to detect. To investigate further, I would conduct longer observations of flocks, and perhaps conduct some form of assay on individual males to elicit a wider range of behaviours rather than just relying on passive observation, as in Roth *et al.* (2021), where the scores of male junglefowl in personality assays was found to be associated with pre- and post-copulatory reproductive success.

In addition, I would measure some physiological traits such as body mass, comb size, growth rate and ejaculate traits such as sperm count and sperm motility. Previous studies have suggested that body conformation can impede effective sperm transfer in some broiler breeder males, with some authors finding that pelvis width and breast angle are negatively associated with fertility (Barbato 1999; McGary, Estevez, and Bakst 2003). Determining whether any birds test positive for avian diseases could also be valuable, since these can influence reproductive success. However, in this scenario the birds were housed on extremely high biosecurity farms, and so pathogen exposure will have been low.

Very many traits are associated with male reproductive success. However, it is often not known *how* they affect reproductive success. Does the effect come about via an influence on the outcome of competition, or would the trait affect reproductive success in the absence of competition? And through which component of male reproductive success does the association occur- through mating success, paternity success, or partner fecundity? We have not identified any relevant trait in this chapter, however, we have determined that there is an opportunity for traits to affect reproductive success at several points: both competitive and non-competitive, via mating success, to a possibly lesser extent via paternity share, and very little via partner fecundity. We find that the addition of male intra-sexual competition creates opportunity for selection via mating success and paternity share, but does not seem to create opportunity for selection via partner fecundity.

Chapter 4: Patterns of mating behaviour and fertility in commercial broiler breeder flocks

"The dunghill cock and hen, in a natural state, pair. In a domestic state, however, the cock is a jealous tyrant, and the hen, a prostitute."

- William Smellie, *The Philosophy of Natural History* (1779)

Abstract

There is very little research into sexual behaviour of broiler breeders in a commercial environment; most behavioural research in broiler breeders uses experimental flocks that are at least one order of magnitude smaller than commercial flocks. This is despite the fact that i) understanding the sexual dynamics of broiler breeders in the environment in which they actually live is of great economic significance and ii) since chickens are the most numerous bird species, and a large proportion of them live in a commercial setting, the behaviour of commercial broiler breeder flocks is of inherent scientific interest. This chapter provides an exploratory analysis of the sexual behaviour and fertility of commercial broiler breeder flocks, observing the behaviour of flocks on four UK farms (sixteen flocks total), and focussing on one farm (four flocks) to investigate variation in fertility. Our behavioural results include the findings that copulation frequency shows strong diurnal variation, and declines with age; that stochastic variation exists between flocks; and that copulation rates, and the success rate of attempts, are influenced by female resistance, which itself shows diurnal patterns of variation. Our results in terms of fertility confirm previous findings that fertility declines with age. We do not detect significant between-flock variation in fertility.

Contributions

The chapter was conceived by Tommaso Pizzari and Grant McDonald. Data was collected by Ellen Pasternack. Data analysis was carried out by Ellen Pasternack, with input from Tommaso Pizzari, Grant McDonald, and Eleanor Bath. The chapter was written by Ellen Pasternack, with input from Eleanor Bath, Tommaso Pizzari, and Grant McDonald.

Introduction

In the eighteenth century, Scottish encyclopaedist William Smellie noted that the sexual behaviour of fowl differs between natural and domestic settings (see epigraph to this chapter)- although he was wrong, however, that the natural state of fowl is monogamy. Today, we have a much more detailed understanding of sexual behaviour and determinants of reproductive success in this species in a natural setting, based on studies in the wild, in zoos, and in naturalistic research aviaries (Collias and Collias 1967; Mcbride, Parert, and Foenander 1969; McDonald et al. 2017; Pizzari and McDonald 2019). In the wild, populations of red junglefowl (*Gallus gallus ssp*.) live in groups of up to around 30-40 individuals in an environment of dense jungle undergrowth. Groups exhibit fission-fusion dynamics, and usually consist of a dominant male, associated females and their offspring, plus some lower-ranking satellite males, with a polygynandrous mating system. When domestic chickens become feralised, they revert to a similar system of social organisation (Collias and Collias 1967; Mcbride, Parert, and Foenander 1969; Pizzari and McDonald 2019).

Today, however, most individuals of this species are found in a commercial setting, where they may live in flocks made up of thousands of birds, in a very crowded and relatively featureless environment. In broiler breeders, where adult males and females are kept together with the aim of producing fertile eggs, the typical flock will consist of anywhere from around two thousand to ten thousand birds, at a stocking density of approximately five birds per square metre (De Jong, Wolthuis-Fillerup, and Van Emous 2009). Generally, broiler breeders live in barns furnished with nest boxes and ad libitum water drinkers on a raised slatted area, and a floor covering of wood shavings, which when combined with accumulated droppings gives a semi-friable earth-like texture. Additional environmental enrichment, such as furnishings that can be used for perching, is usually minimal (Leone and Estévez 2008). Further departures from the natural environment occur in the feeding regime, in which the entire food intake is consumed at one point in the day, and in the absence of heterospecifics, including predators, vigilance for which is an important part of the social ecology of this prey species (Wilson et al. 2008). There are many ways in which we might expect all these environmental changes to affect the socio-sexual dynamics of broiler breeders (Garnham and Løvlie 2018; Pizzari 2016).

The commercial purpose of broiler breeders is to produce fertile eggs, and due to the pyramidal breeding structure of the industry, even marginal changes in fertility could have large implications for efficiency. However, unlike many physiological traits which have been enormously improved by selective breeding, fertility itself is only moderately heritable, being determined in large part as the outcome of social interactions (Barbato 1999). Over the years, improvements in the hatchability of broiler breeder eggs has plateaued (Laughlin 2008). In addition, many lines are plagued with low fertility, and fertility in all lines declines as birds age. The fact that low fertility (in problem lines, or in older birds) can often be remedied by artificial insemination suggests that this is not a physiological problem but one of behaviour (Laughlin 2008). Further supporting this view is the finding that sperm quality poorly correlates with fertility on an individual level (Bilcik, Estevez, and Russek-Cohen 2005). Understanding the behaviour of these birds in a commercial setting is therefore of great importance.

Rationale for this chapter

Early studies on behaviour tended to focus on domestic chickens living in naturalistic environments: natural group sizes and less extreme density (Guhl, Collias, and Allee 1945). Studies of the behaviour of broiler breeders typically use experimental pens of at most around 150 birds, and often much fewer (Bilcik, Estevez, and Russek-Cohen 2005). This is a group size at least one order of magnitude smaller than the flocks in which broiler breeders are kept commercially. We therefore have comparatively little understanding of the behavioural dynamics of broiler breeders in the environment in which they actually live. In addition, the effect that behavioural dynamics might have on flock fertility is almost totally unknown, despite being of huge significance economically.

This chapter aims to provide an exploratory investigation into the determinants of copulation rate in commercial flocks of broiler breeders, and into inter-flock variability in copulation rate and fertility. Four flocks are studied on each of four farms (giving sixteen flocks in total), allowing for the detection of differences that may be attributable to varying husbandry practices or other local differences between farms as distinct from random variation (e.g. differences between flocks on the same farm).

In this chapter I will investigate:

i. How copulation rate varies between old and young birds, between different flocks, and between observations taken at different times of day

ii. Whether any differences that are detected between young flocks persist as birds age

iii. Which behaviours are associated with high or low copulation rate

iv. How female resistance to copulation varies between old and young birds, between different flocks, and between observations taken at different times of day, and how it acts to affect copulation rate

v. Whether fertility varies between flocks, and whether variation in fertility reflects variation in copulation rate

Sexual behaviour in large groups is a complex, interacting system. Behaviours may be influenced by feedback loops that amplify small initial differences. For instance, sexual harassment by males can cause harm to females, and promote increased female resistance to mating attempts or spatial avoidance of males. This would increase the operational sex ratio and may therefore prompt males to redouble their efforts in sexual harassment (Tommaso Pizzari 2016). Another example could be increased mating activity in the population prompting males to increase their own reproductive effort due to increased perceived levels of sperm competition (Birkhead and Møller, 1992; Pizzari *et al.*, 2003), again potentially causing small initial differences to be amplified. Environmental enrichment in flocks of laying hens during rearing can alter their behaviour later in life, decreasing the incidence of floor eggs and cannibalism (I. Estevez 2009), showing that group behaviour is plastic and can follow different trajectories depending on seemingly small inputs. It is therefore possible that there may be noticeable stochastic variation between flocks in group-level behavioural measures. However, the extent of these differences has not been characterised before.

The behaviour and fertility of broiler breeder flocks in a commercial setting is expected to differ significantly from either junglefowl or traditional lines of domestic chickens in a naturalistic setting. This is for reasons relating both to the phenotypic changes wrought by intense artificial selection and to the unique socio-sexual environment provided by broiler breeder farms. Some of these relevant differences and their potential effects on sexual behaviour and fertility are detailed in the sections below.

Effects of artificial selection on sexual behaviour of broiler breeders

Compared to traditional lines of domestic chickens, today's broiler breeders have been subject to decades of intensive selective breeding, in particular for large size, rapid growth rate, and high breast yield, i.e. large pectoral muscles (Havenstein, Ferket, and Qureshi 2003; Zuidhof et al. 2014). Very large phenotypic changes have occurred within a brief evolutionary timeframe, leaving little opportunity for supportive traits, such as stronger bones and more powerful hearts to support larger frames, to evolve. In addition, there is a genetic correlation between body weight and leg defects; selection regimes that do not explicitly protect leg health are likely to result in defects as a consequence of selection for body weight (Kapell et al. 2012).

The aim of much of this artificial selection is to produce in phenotypic changes in broilers, rather than broiler breeders. However, they are part of the same lineage and as such have experienced this same extreme selection pressure, with potential implications for their development and behaviour. Artificial selection on broiler breeders may impact sexual behaviour via a decrease in activity levels and locomotor ability (M S Dawkins and Layton 2012), and an increase in the prevalence of musculoskeletal disorders (Paul M Hocking and Duff 1989).

However, there is not a clear negative relationship between individual weight and copulation rate; in fact, there is some evidence that heavier males copulate more (I. J.H. Duncan, Hocking, and Seawright 1990; P. M. Hocking and Bernard 1997). Individual weight *per se* may therefore be only weakly linked to the population-level problems associated with selection for rapid growth (M S Dawkins and Layton 2012).

Instead, specific physiological traits may make the act of copulation more difficult and result in more failed copulation attempts. In turkeys, high breast yield resulting from strong artificial selection is reported to cause such an obstacle to mating that breeders have been forced to resort to artificial insemination (Carte and Leighton 1969; Nestor and Brown 1968). In broiler breeders, a strain with higher breast yield was found to have a lower frequency of observed copulations and a higher proportion of failed attempts (P. M. Hocking and Bernard 1997). In addition, McGary, Estevez and Bakst (2003) found that the fertility of males in a subfertile line of broiler breeders was negatively correlated with pelvis width. A negative association between pelvis width and fertility was also detected by Wilson *et al.*, (1979). Selection for commercially desirable weight distributions may be associated with changes to skeletal conformation which could impede copulation.

In addition, sexual behaviour and fertility may be affected indirectly, via correlated traits. For instance, some studies find lower sperm motility in heavier broiler breeder males (B Bilcik, Estevez, and Russek-Cohen 2005; Bowling et al. 2003). However, Wilson *et al.*, (1979) find that although weight had a negative relationship with fertility in a natural mating setting, this relationship was not found when artificial insemination was used. This and other reports that low fertility can be remedied through artificial insemination suggest that behavioural traits, rather than ejaculate traits, are the main drivers of low fertility in broiler breeder males.

Broiler breeder farms: a unique socio-sexual setting

Group size

Large group sizes may mean that birds are unable to form stable dominance hierarchies, since they do not have the capacity to recognise and recall social information about thousands of individuals (Pagel and Dawkins 1997). Dominance hierarchies allow individuals to predict the outcome of conflicts and make engaging in aggression unnecessary: high levels of aggression when birds are introduced to each other typically decrease as a stable hierarchy is formed. We might therefore expect that commercial flocks would see high levels of aggression. However, it seems that, with incompletely formed dominance hierarchies in large groups, chickens do not respond by showing indiscriminate aggression but rather by increasing social tolerance towards unfamiliar individuals (D'Eath and Keeling 2003; Inma Estevez, Keeling, and Newberry 2003; Inma Estevez, Newberry, and De Reyna 1997).

Such an "incomplete" dominance hierarchy might influence mating behaviour via several routes. In smaller groups of both junglefowl and domestic chickens, dominant social status (predicted by high levels of intra-sexual aggression) is one of the few traits that has been very clearly and consistently linked to male mating success. Females are more likely to socially associate with dominant males, more likely to solicit copulations and less likely to resist copulations from dominant males (McDonald et al. 2017). In large groups, a limited ability to form a clear dominance hierarchy and recall the social position of others, may reduce the influence of social status on male mating success, and might instead increase female reliance on other cues for mate choice.

In addition, independent of their effect on dominance hierarchies, large group sizes may increase the potential for mate choice and increase levels of polyandry (and hence levels of sperm competition), simply by virtue of individuals having more potential mating partners available. Thus, large groups may promote higher rates of copulation in males due to the presence of sperm competition, and due to last male precedence favouring frequent copulation (Birkhead and Møller, 1992).

It is not clear, however, how much social subdivision there is within these large flocks. Incomplete mixing of the population might mean that functional group size is much smaller, and birds can recognise and form hierarchies with the individuals they interact with in their local area. Because the natural state of this species involves territoriality by males, it was long assumed that movement within flocks would be low as it would be restricted by the territories of conspecifics (Duncan 2008; Mcbride, Parert, and Foenander 1969; Pizzari and McDonald 2019). We know that broiler breeders do not tend to space themselves evenly in the area available to them, instead clustering round the edges and any sheltered areas, which might indicate a low level of mixing. However, the evidence on movement patterns and space use in commercial chickens does not tend to support the idea of functional subgroups, including in broilers (Leone and Estevez 2008) and in free-range laying hens (Rodriguez-Aurrekoetxea and Estevez 2016). A tracking experiment in flocks of 4,000 birds by (Appleby, Maguire, and McRae 1985) found that the median bird's range covered 73% of the available area, indicating large overlapping of ranges. In addition, no difference was detected between the ranges of males and females.

Environmental uniformity

Behavioural ecology research in a wide variety of species tells us that environmental complexity affects the expression of sexual behaviours. Environmental complexity gives individuals the opportunity to escape from aggression, including sexual aggression and forced copulation (Perry and Rowe 2018). It can decrease the ability of dominant males to monopolise mating opportunities, by allowing subordinate males to copulate without attracting attention (Perry and Rowe 2018). It is therefore possible that the simple environments in which broilers live might lead to increased monopolisation, increased variance in male mating success, and decreased levels of polyandry, relative to more complex naturalistic environments. Monopolisation of mating opportunities by a small number of males may lead to females being sperm-limited and showing incomplete fertility. The opportunity for females to exert precopulatory mate choice is likely limited, because of decreased ability to avoid forced copulations, and engage in covert copulations with preferred males (Dean et al. 2010).

In addition, the low environmental complexity of broiler breeder farms may affect sexual behaviour via proximate behavioural mechanisms. In farmed animals, low-complexity environments can induce stress and behavioural stereotypies, and thus may decrease the ability to engage in "normal" sexual behaviour (Estevez 2009).

The effects that low-complexity environments may be having on the sexual behaviour and fertility of broiler breeders are suggested by experiments investigating the effects of adding "cover panels" and other forms of environmental enrichment. "Cover panels" refer to two-dimensional frames placed vertically to provide partial barriers (visual and spatial) in broiler breeder sheds or enclosures. Other forms of environmental enrichment include perches, ramps, litter (as opposed to a barren floor), hanging objects as a target for pecking, and even coloured lights projected onto the floor (Bizeray et al. 2002; Estevez 2009).

Leone and Estévez (2008) find that adding cover panels increases the number of eggs laid, and the fertility and hatchability of eggs. However, since no difference was detected between mesh panels and "frame only" panels, it seems that it is not just their role as visual barriers that means cover panels have this effect. Cover panels provide desirable sheltered sites that attract broilers, encouraging birds to make use of the whole available space and decreasing clustering of birds around the edges of pens (Cornetto and Estevez, 2001). This decreases conflict and aggression over other desirable sites (Cornetto, Estevez, and Douglass 2002) and is likely to reduce stress and increase activity levels (Cornetto and Estevez, 2001), and may therefore increase fertility in broiler breeders by increasing the encounter rate between potential mating partners (although evidence on this is mixed- Leone and Estévez (2008) find home ranges of male broiler breeders are increased in the presence of cover panels, but Estevez *et al.* (2010) find no effect on total distance moved when cover panels were added). These differences in behaviour when minimal enrichment is added suggests that in the standard pens, copulation rate may be suppressed by low environmental complexity which causes increased stress, increased aggression, and less effective movement.

Feeding regime

In nature, the food that makes up the junglefowl diet (i.e. largely vegetable matter and small invertebrates) is dispersed through the environment. Much of the daily activity of junglefowl and feral populations of domestic chickens is therefore concerned with foraging (Dawkins 1989); individuals eat fairly continuously over the course of the day and are unlikely to experience large daily fluctuations in hunger levels.

The situation for broiler breeders could hardly be more different. Since broilers have been selected to grow extremely fast, these birds have large appetites and are highly motivated to seek food. However, unlike broilers, broiler *breeders* are not provided with ad-libitum feed; instead, they are fed once daily with a calorific intake calculated to maintain a healthy weight (Hocking, Bernard, and Robertson 2002). Therefore, these birds are likely not only to exist in a state of hunger but to experience large fluctuations in hunger levels over the course of a day due to their intake of food being restricted to a single point in time rather than throughout the day.

Mating behaviour follows a clear circadian rhythm in fowl, with a peak in mating activity in the evening (Bilcik and Estevez 2005; Løvlie and Pizzari 2007). Fertility also shows a circadian pattern, with the chances of fertilisation being low in the morning before an egg is laid (since the hard-shelled egg blocks sperm from travelling up the oviduct), and a fertile window later in the day after the egg has been laid (Birkhead and Møller, 1992; Bakst, Wishart and Brillard, 1994). The ejaculates of males may by tailored to this cycle, with males producing greater ejaculate volume and greater absolute numbers of sperm in the evening than in the morning or the middle of the day (Lake and Wood-Gush 1956). Extreme fluctuation in hunger levels in broiler breeders could have the potential to disrupt the normal circadian rhythm of mating behaviour, and its alignment with fertility.

Mating behaviour is typically influenced by levels of hunger, with hungry individuals less motivated to mate (except in species with nuptial feeding, where hunger can increase female receptivity) (Ando, Yoshimizu, and Matsuo 2020; Rowe 1992). It is possible that fluctuating levels of hunger throughout the day could lead to peaks and troughs in copulation rate throughout the day that are not seen in other fowl populations living under more natural conditions. Additionally, it is possible that the general high levels of hunger that broiler breeders experience could depress copulation levels in general.

Uniform age distribution

Another unusual feature of the commercial poultry environment is that groups comprise of birds from the same cohort, meaning there is no stratification in age. In junglefowl and feral fowl, age strongly influences male reproductive success, with younger males favoured in both pre- and post- copulatory stages (Carleial et al. 2020; Dean et al. 2010; McDonald et al. 2017). In addition, although the association between age and social status in males is weak, age is a key determinant of dominance hierarchies for females, with older females more likely to occupy a dominant social role and therefore to have greater reproductive success (McDonald et al. 2019).

The lack of age stratification in broiler breeder flocks may therefore have the effect of flattening inter-individual variation in reproductive success, or mean that random chance explains a greater proportion of variance in reproductive success. It may also mean that mate choice is less important: if all individuals are more similar to each other, then there is less scope for judicious mate choice to affect an individual's fitness.

Methods

Behavioural observations

Birds at four farms (farms A, B, C and D) were observed between January 2019 and July 2021. All farms were located in Lincolnshire, UK, and owned and operated by Moy Park, a UK-based poultry meat producer. All birds were of the same breed (Ross 308) and the same lineage was present on all four farms.

Each farm contained 4 – 8 “houses”: long barns of approximately 70m x 20m, housing approximately 6,000 birds. Each house is divided down the middle into two long halves each of approximately 70m x 10m, with birds almost exclusively confined to one half. At each farm, I arbitrarily selected four houses, and observed within one half of each of these houses.

Observations took place at three different times of day: “morning”, which was between the farm opening in the morning and the birds being fed (around 06.30 – 08.30); “post feed”, which began half an hour after the birds’ daily food ration was distributed (around 09.30 – 11.30); and “evening”, which was within the final two hours before the birds’ lights were switched off (around 15.00 – 17.00). In nature, the birds’ evening peak in mating activity would be later than this; however, under artificial light:dark schedules a peak is seen in the hours immediately before lights out, after which birds soon go to roost.

There was some small variation in these timings between farms, but observations remained consistent with respect to the landmarks of farm opening, feeding, and lights off. For each farm, observations took place on either four or five consecutive days, with the order of houses changed each day for a balanced design.

The units of observation were twenty-minute sessions, timed with a stopwatch. Up to six observations were taken for each house at each of the three time of day (with some variation caused by farm timetables and other practical constraints).

Houses on all four farms followed the same design, with a raised bank of nesting boxes along one long edge, and regularly spaced beams supporting the roof. During each twenty-minute observation session, I sat on the edge of the bank of nesting boxes and counted behaviours that occurred in the area between the two beams on either side of me (area approximately 4m x 7m). Each observation within one house is taken at a different randomly chosen observation point. The number of birds was not directly controlled for as this would have been prohibitively difficult, with large numbers of individuals involved and movement in and out of the sampled transect. Each sample is considered to be roughly equivalent as we have no a priori reason to believe that any one transect will contain more birds than another.

A description of the behaviours that were recorded can be seen in ***Table 1***. Occurrences of each behaviour within the sampling area during the twenty minute sample were tallied. In the case of female resistance, female behaviour for each copulation or male copulation attempt was classified as either resistant or non-resistant (see ***Table 1*** for description). The proportion of male attempts that were resisted can then be calculated for each sample.

Initially, the plan was to observe flocks on four different farms when the birds were "young" (25-30 weeks of age, which is the age of peak fertility for broiler breeders) and again when "old" (48-53 weeks of age, close to the end of their lives). However, due to unavoidable disruptions I was not able to collect all the data as planned. All four farms A-D were observed when flocks were young, but only flocks in Farm A and Farm B were observed again when they were old. The birds on farm C were culled early due to an outbreak of disease, meaning they could not be observed again when they were old. In addition, work was disrupted by national coronavirus lockdowns throughout 2020 and early 2021, and then by farm visiting restrictions due to avian influenza winter 2021-2022.

Table 1: *Ethogram showing the behavioural measures that were recorded for each observation session.*

Behavioural measure	Description
Successful copulation	A successful copulation is determined by cloacal contact between male and female, either directly observed or inferred from the positioning of the male train over the female cloaca. It is possible that some cases where cloacal contact was inferred were in fact unsuccessful, as it is not possible to tell whether cloacal contact definitely occurred when obscured from view by the male's tail, but the error should be in only one direction, i.e. no successful copulations should have been missed as a result of this classification system.
Male copulation attempt	An attempt was counted if any of the following was observed: • Male approaches female from behind with hackles raised • Male grasps female's comb or head/neck feathers in his beak • Male places one foot on the back of a female

	- Male mounts the female with both feet on her back
Mount	Male mounts the female with both feet on her back
Courtship	An instance of courtship behaviour was counted if a male was seen making a food call (stereotyped pattern of picking up an item in his beak) or waltzed at a female (turned in an arc in front of her with outer wing outstretched and beating against leg)
Aggression (female)	Females face each other with necks upstretched and aim pecks at each other's heads.
Aggression (male)	All instances of aggression between males including pecking, chasing, or lunging at another male, waltzing (as in Courtship, but between males), or interruptions of mating attempts.
Solicitations received	A solicitation was recorded when a female was seen crouching in front of a male to invite copulation
Female resistance	A female resists a mating attempt if she moves away from a male's mating approach, or when grabbed is seen to struggle or audibly utters a distress call. If none of these is observed, she is said to have accepted the attempt. For each male-initiated attempt, whether the female accepted or resisted the attempt was recorded.

Fertility assessment

Thirty-five eggs were collected from nest boxes within each of the four houses on Farm D when the birds were young, and refrigerated on the day of laying. Thirty-five more eggs were collected from each house when the birds were old. Since eggs are collected from the houses every day, it is highly likely that each of the thirty-five eggs per sample came was laid by a different female.

The fertility of each egg was assessed both by visual inspection of the germinal disc and by counting the number of sperm hydrolysis points in the perivitelline membrane surrounding the yolk. The number of sperm hydrolysis points is taken to indicate the number of sperm present in the infundibulum at the time the ovum is released, and decreases logarithmically with time since insemination, reflecting sperm loss from the female sperm storage tubules (Bakst, Wishart, and Brillard 1994). Importantly, the number of sperm hydrolysis points correlates with the probability of fertile appearance of the germinal disc (Bramwell et al. 1996; Brillard 2003; Wishart 1987).

Each egg was opened, and the appearance of the germinal disc located on the yolk membrane classified as either fertile (a perfectly round white dot with a defined edge, about 5mm in diameter) or not fertile (a white speck, unevenly shaped, and about 2mm in diameter) (Kosin 1944).

Next, a square of perivitelline membrane approximately 20x20mm was cut around the germinal disc, and washed sequentially in two dishes of phosphate-buffered saline to remove yolk residue. This square of membrane was placed on a microscope slide and viewed at 10x magnification. In most cases a characteristic “scattergun” cluster of holes in the membrane could be readily found (Hazary, Staines, and Wishart 2000; Wishart 1987). These were counted by placing the centre of the cluster in the bottom left of the microscope view, counting all visible hydrolysis points using a clicker counter, and then repeating this with three more microscope views, moving clockwise around the centre of the cluster. In cases where a cluster was not identified, a zero count was recorded if no hydrolysis points were located after systematically viewing the entire square of membrane via horizontal and then vertical sweeps.

These measures of fertility (germinal disk inspection and the number of sperm hydrolysis points on the perivitelline layer) are not influenced by early embryo mortality, which makes up a sometimes large “invisible fraction” of eggs deemed infertile due to hatching failure or infertile appearance when candled at a later stage of development (T. R. Birkhead et al. 2008; Nicola Hemmings and Evans 2020).

Statistical analysis

i) Characterising variation in copulation rate

Copulation rate was modelled using the negative binomial distribution, first for both old and young birds together with a fixed effect for age (young or old), and then for young and old datasets separately, to account for expected behavioural differences between young and old birds. House was added as a random intercept, but farm was treated as a fixed effect as this improved model fit (treating farm as a random intercept gave significant deviation according to Kolgorov-Smirnov test (p = 0.024, Berger and Zhou, 2014). Neither dispersion parameters nor zero inflation parameters were required for acceptable model fit. Model specifications are detailed in ***Table 2***. In these models, each twenty-minute observation represents one data point.

Table 2: *Model specifications for characterising variance in copulation rate, for young and old birds.*

Time point	Full model
Young + Old	`Copulations ~ Time + Age + Time * Age + Farm + (1|House),` `family = nbinom1()`
Young only	`Copulations ~ Time + Farm + (1|House),` `family = nbinom1()`
Old only	`Copulations ~ Time + Farm + (1|House),` `family = nbinom1()`

These models were fit using the R package *glmmTMB* (Brooks *et al.*, 2017). All models were checked for outliers, dispersion, zero-inflation, and evenness of residuals using simulation-based tests in the package *DHARMa* (Hartig 2020). The Kolgorov-Smirnov test was used to test for acceptable goodness of fit (Berger and Zhou 2014).

To test for the significance of individual variables, each variable was removed in turn from the full model, and a likelihood ratio test conducted between the full model and the reduced model (Lewis, Butler, and Gilbert 2011).

All subsequent analyses for aims (ii, iii, and v) focus on evening observations, as opposed to other times of day, because we found the highest rate of copulation at this time, and because copulations in the evening have the greatest biological relevance, having the highest chance of leading to fertilisation (Lake and Wood-Gush, 1956; Birkhead and Møller, 1992; Bakst, Wishart and Brillard, 1994).

ii) Relationship between copulation rate when young and when old

At Farm A and Farm B, behavioural observations were carried out at both “young” and “old” time points. This gives eight houses with values for mean evening copulation rate at both time points.

To test whether a flock’s copulation rate when young could predict copulation rate of the same flock when old, confidence intervals were calculated for the Pearson’s correlation coefficient between the mean number of copulations observed per house during evening observation sessions at the “young” and the “old” time point. Confidence intervals were calculated using the R package *confintr* (Mayer 2022). A relationship was detected if the confidence interval for the correlation coefficient did not overlap 0.

iii) Associations between copulation rate and other behaviours

To test which other behaviours were associated with the rate of copulation in evening observation sessions (see ***Table 1*** for list of behaviours), linear mixed effects models were fit using the package *lme4* (Bates et al. 2015). The Normal distribution is acceptable for use with count data in this case because when the value of observations is high, distributions of count data approximate a Normal distribution (continuous and symmetric rather than discontinuous and curtailed at zero) (Smith and Skrakowski 2019). Values for the number of copulations were sufficiently high that model fit was found to be acceptable by simulation-based tests in the package DHARMa (Hartig 2020).

The general null model included age ("young" vs "old"), farm, and their interaction as fixed intercepts, and house as a random intercept. As before, model fit was much better if farm was not treated as a random effect (Δ AICc = 25); model fit was also substantially improved by including the interaction between age and farm (Δ AICc = 10). For each behavioural measure, a model that included that measure and an interaction between that measure and age was compared to the null model via likelihood ratio test.

For the number of mounts and the total number of attempts, the number of successful copulations was subtracted, so that these become counts of unsuccessful mounts and unsuccessful attempts to avoid autocorrelation with the number of successful copulations. Additionally, we tested the significance of the "mount rate"- the proportion of attempts that are converted to mounts, with successful copulations removed from both numerator and denominator to avoid autocorrelation.

Since possible values for the proportion of attempts resisted are restricted by the number of attempts that take place, it was decided that any observation sessions with fewer than five copulation attempts would be discounted. However, there were no evening observation sessions meeting this description and so no observations were excluded from the dataset (minimum number of attempts seen = 6).

Table 3: *Model format used to test associations between copulation rate and other behaviours observed.*

Null model	`Copulations ~ Age * Farm + (1|House)`
Alternative model	`Copulations ~ Age * Behaviour + Age * Farm + (1|House)`

These were compared to a null model without any additional behavioural predictors using likelihood ratio tests. The Benjamini-Hochberg procedure was used to correct for multiple comparisons (Benjamini and Hochberg 1995).

iv) Female resistance to mating

As before, the data was restricted to include only observation sessions where at least five mating attempts by males were seen. This is because it was thought that smaller numbers of attempts would not give an accurate estimate of the true population receptivity. Since a majority of morning observation sessions were excluded by this criterion, leaving sparse or missing data for many houses, all morning sessions were excluded from the analysis. In addition, six post-feed sessions (out of 84) were excluded. No evening sessions were excluded by this criterion.

To characterise variance in the level of female resistance, a linear mixed-effects model was fit using the R package *lme4* (Bates et al. 2015), with proportion of attempts resisted as the dependent variable; age (old vs young), time of day (post-feed vs evening), and the interaction between the two as fixed intercepts; and farm and house as random intercepts. Each data point in the model was one twenty-minute observation session, with some sessions excluded as described above. To test the significance of predictors, terms were removed in turn from the model and likelihood ratio tests conducted to compare reduced models to the full model.

Next, the effect of female resistance on different components of copulation success was investigated. To copulate, a male must make a copulation attempt, successfully mount the female, and then achieve cloacal contact (see ***Table 1***). Pooling across all observation sessions, a total of 58% of male attempts resulted in mounts, of which 73% resulted in copulations, giving a total success rate of 42% of male attempts resulting in copulations. Across the observation sessions included in resistance models (no morning observations, and no observations with fewer than 5 male attempts), these figures are extremely similar: 59% of male attempts resulted in mounts, of which 73% resulted in copulations, giving a total success rate of 43% of male attempts resulting in copulations.

Linear mixed-effects models were fit to explore the effect of female resistance on each of these success metrics using *lme4* (Bates et al. 2015) (see ***Table 4***). As before, each data point in the model is one observation session, with some sessions excluded as described above. House was not included as a random intercept, as its inclusion produced singular fit errors, and its exclusion was justified by the absence of any significant effect detected of house on resistance levels. Full models allowed the proportion of attempts resisted to interact with both time of day and age. Likelihood ratio tests between the full models and alternatives shown in ***Table 4*** were used to determine whether effects of resistance were statistically significant.

***Table 4:** Models used to investigate the influence of female resistance on three different metrics of copulation success: conversion of attempts to mounts, conversion of mounts to copulations, and overall success rate (conversion of attempts to copulations). "Resistance" refers to the proportion of male attempts that were resisted by females.*

Full model	`Success ~ Resistance * Time of day + Resistance * Age + (1|Farm)`
No effect of resistance	`Success ~ Time of day + Age + (1|Farm)`
No interaction with time of day	`Success ~ Resistance + Time of day + Resistance * Age + (1|Farm)`
No interaction with age	`Success ~ Resistance + Age + Resistance * Time of day + (1|Farm)`

v) Fertility

Two measures of fertility were used in this study: number of sperm hydrolysis points in the perivitelline layer (PVL) and the fertile appearance of the germinal disc (GD). Generalised linear mixed-effects models were fit to explain each of these as a function of age and house. PVL was modelled using the negative binomial distribution, and a logistic model was used for GD, with each egg being recorded as 1 for fertile appearance and 0 for non-fertile appearance. A zero-inflation term was added to the PVL model to account for high, variable levels of zero-inflation. Full models for PVL and GD are shown in ***Table 5***. To test the significance of age and house as predictors, terms were removed and compared to the full model via likelihood ratio tests (Lewis, Butler, and Gilbert 2011).

***Table 5:** Models used to test significance of age and house in explaining variance in fertility.*

Fertility Measure	Full Model
PVL	`Fertility ~ Age + (1|House), zi ~ Age + (1|House), family = nbinom1()`
GD	`Fertility ~ Age + (1|House),` `family = binomial (link = logit)`

To test whether either measure of fertility showed a relationship with copulation rate, confidence intervals were calculated for the Pearson's correlation coefficient between the mean number of copulations observed per house during evening observation sessions at the "young" time point and both measures of fertility: PVL and GD, at the "young" and "old" time points. Confidence intervals were calculated using the R package *confintr* (Mayer 2022). A relationship was detected if the confidence interval for the correlation coefficient did not overlap 0.

Results

i) Characterising variance in copulation rate

Time of day was a strong predictor of copulation rate (χ^2 = 408.62, df = 4, p < 0.001). Large differences were observed in copulation rate at each of the three times of day tested (Cohen's d, evening vs morning: 2.29; evening vs post-feed: 1.24; morning vs post-feed: 1.99). Copulation rate was highest in the evening, followed by post-feed, with morning rates of copulation the lowest.

There was a significant decrease in copulation rate in "old" birds compared to "young" birds (χ^2 = 57.678, df = 3, p < 0.001), and a significant interaction between age and time of day (χ^2 = 6.014, df = 2, p = 0.049): "old" birds decreased their copulation rate particularly in the evening. There were significant differences in copulation rate between houses (χ^2 = 12.199, df = 1, p < 0.001) and between farms (χ^2 = 16.495, df = 3, p = 0.001).

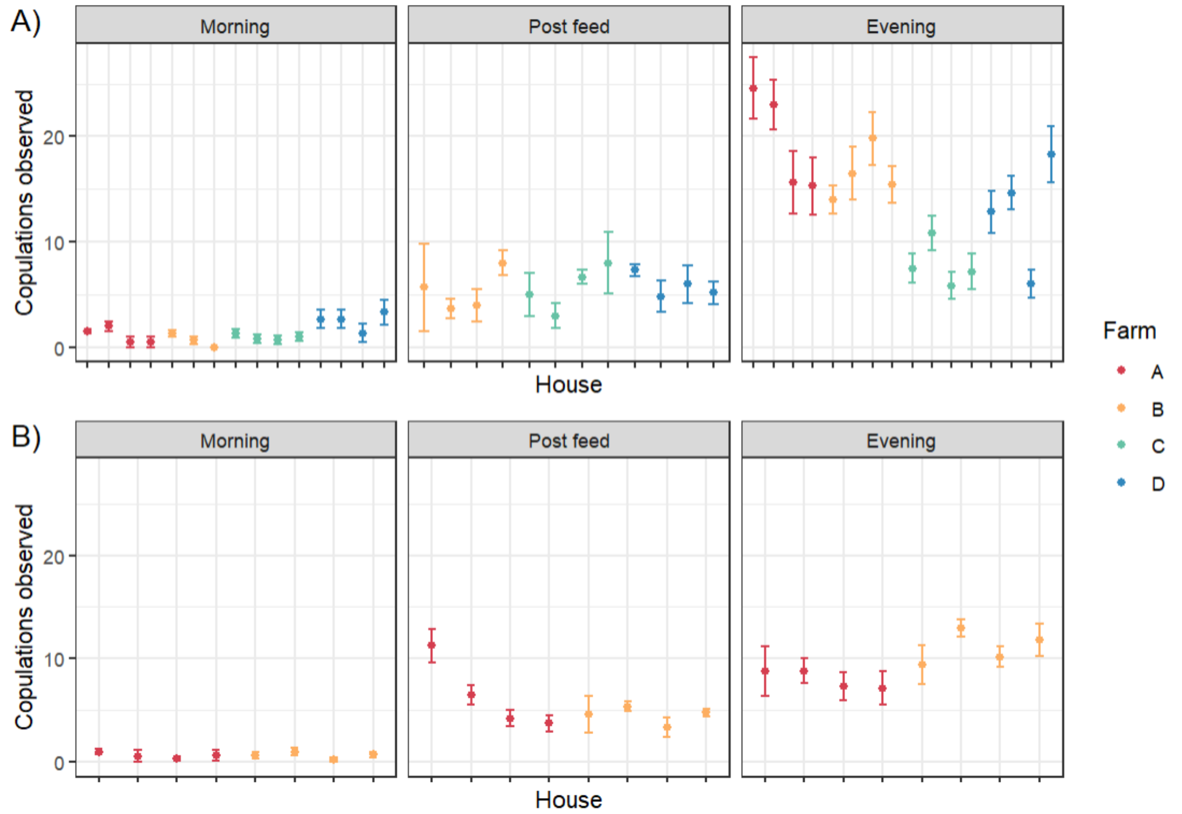

Figure 1: *Observed copulation rates in birds across four houses at four farms per twenty minute observation. Each point represents the mean value for one house, with houses shown in the same order across the three panels. Error bars represent standard error.* ***A)*** *shows observed values when birds were young, and* ***B)*** *shows observed values when birds were old, for the two farms that were also observed at this time point.*

The majority of these effects were confirmed when “young” and “old” birds were analysed separately. Copulation rate varied by time of day for both young (χ^2 = 252.69, df = 2, $p < 0.001$) and old birds (χ^2 = 157.37, df = 2, $p < 0.001$) (likelihood ratio tests between full models including time of day as a fixed effect and reduced models with these variables removed). Again, large differences were observed between each of the three time points for both “young” (Cohen’s d, evening vs morning: 2.30; evening vs post-feed: 1.35; morning vs post-feed: 1.87) and “old” birds (Cohen’s d, evening vs morning: 3.07; evening vs post-feed: 1.14; morning vs post-feed: 2.19). Post-hoc testing using Wilcoxon rank tests with Benjamini-Hochberg-corrected p values confirmed statistically significant differences between the copulation rate at each time of day for both young and old flocks ($p < 0.0001$ for every pairwise comparison).

Similarly, both farm (χ^2 = 15.79, df = 3 p = 0.001) and house (χ^2 = 6.784, df = 1, p = 0.009) explained a significant amount of variance in copulation rate (likelihood ratio test between full model, and reduced models with either of house or farm removed) in “young” birds.

For old birds, including house and farm as predictors no longer has a significant effect on model likelihood (Farm: χ^2 = 0.959, df = 1, p = 0.372; House: χ^2 = 1.582, df = 1, p = 0.209; likelihood ratio tests between full models and reduced models with these variables removed). These patterns for “young” and “old” birds are visualised in ***Figure 1***.

ii) Does a house’s copulation rate when young predict the copulation rate when old?

No relationship was detected between the mean number of copulations observed in the evening at the “young” and “old” time points (95% confidence interval for Pearson’s correlation coefficient: -0.75, 0.65; ***Figure 2***).

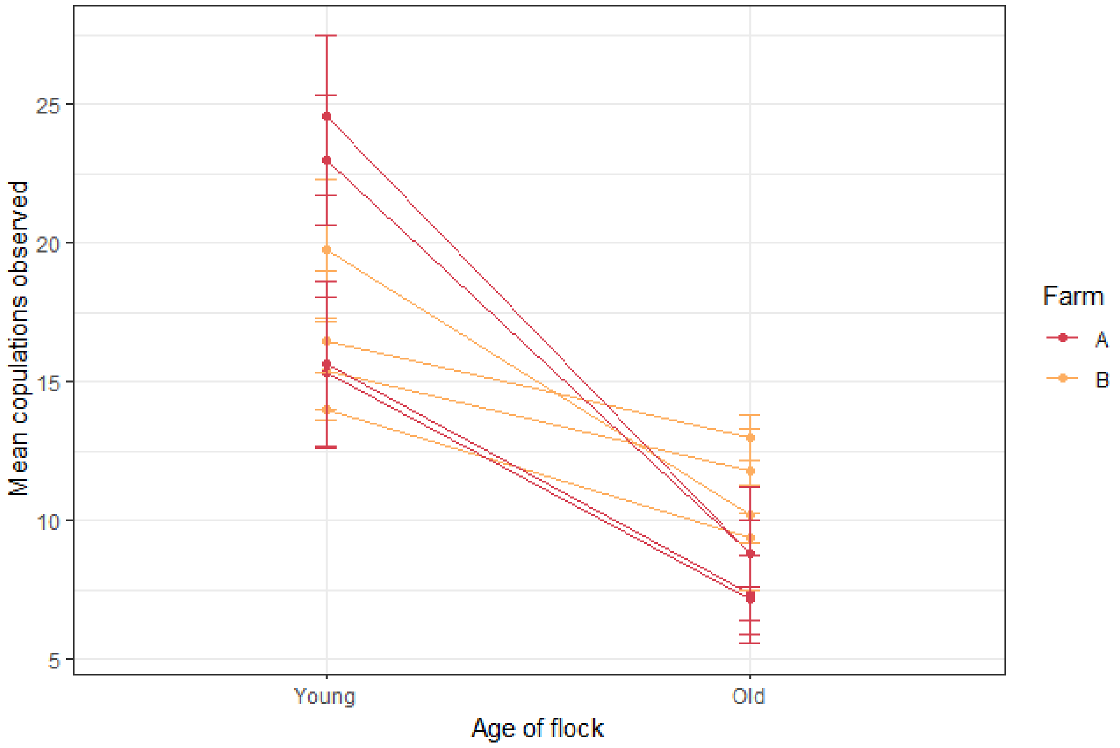

Figure 2: *Copulation rate when young and when old, for houses at the two farms for which data were available at both time points. Each point on the graph represents the mean number of copulations seen in evening observation sessions for one house at either the young or the old time point. Observations at the same house across time are linked by lines. Error bars represent standard error.*

iii) Which behaviours are associated with copulation rate?

A positive association was detected between copulation rate in the evening and number of unsuccessful attempts, the number of unsuccessful mounts, and the proportion of unsuccessful attempts resulting in mounts by males. A negative association was detected between copulation rate in the evening and the proportion of attempts that were resisted by females. No association was detected with courtship, male-male or female-female aggression, or the number of solicitations made by females. These relationships are visualised in ***Figure 3***. Test statistics and p-values are given in ***Table 6***.

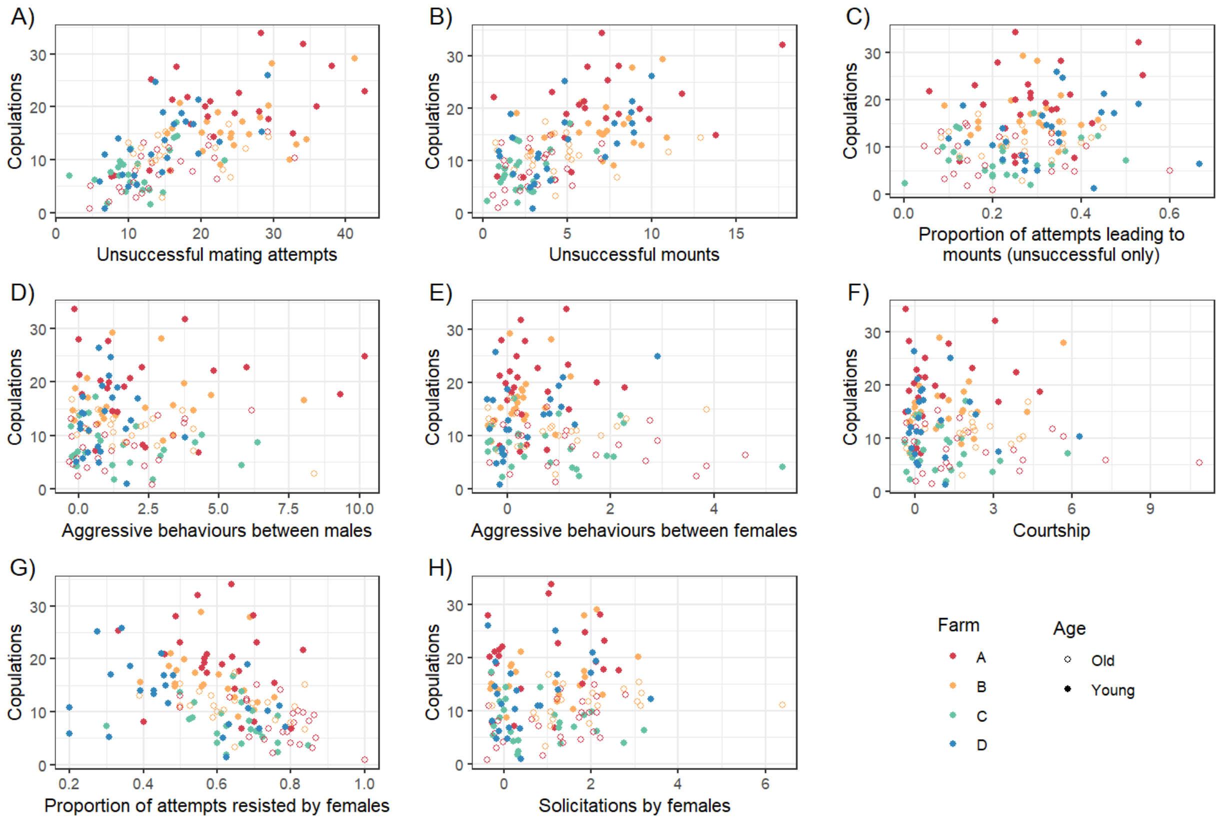

Figure 3: *Relationship between copulation rate and other observed socio-sexual behaviours. Each point on the graphs represents the result of one twenty-minute observation session during the evening.*

***Table 6:** Test statistics for associations between copulation and other behavioural measures (as defined in **Table 1**).*

Behavioural measure	Test statistic	p-value adjusted for multiple comparisons
Attempts that did not result in successful copulation	$\chi^2 = 27.106$, df = 2, $p < 0.001$	$p < 0.001$
Mounts that did not result in successful copulation	$\chi^2 = 23.943$, df = 2, $p < 0.001$	$p < 0.001$
Mount rate	$\chi^2 = 14.387$, df = 2, $p < 0.001$	p = 0.002
Courtship rate	$\chi^2 = 0.618$, df = 2, p = 0.734	p = 0.734
Male-male aggression	$\chi^2 = 0.737$, df = 2, p = 0.692	p = 0734
Female-female aggression	$\chi^2 = 3.823$, df = 2, p = 0.148	p = 0.197
Proportion of attempts resisted by females	$\chi^2 = 20.439$, df = 2, $p < 0.001$	$p < 0.001$
Female solicitations	$\chi^2 = 5.055$, df = 2, p = 0.080	p = 0.128

iv) Characterising variance in female resistance to copulation, and its influence on copulation success

Significant differences were detected between resistance levels at different times of day ($\chi^2 = 62.651$, df = 2, $p < 0.001$). Resistance in post-feed sessions was lower than in the evening (Cohen's d: 1.20). No difference in the level of female resistance was detected between young and old birds ($\chi^2 = 5.491$, df = 3, p = 0.139). The random intercept of farm was found to have a significant effect on model likelihood ($\chi^2 = 8.882$, df = 1, p = 0.003), but house did not ($\chi^2 = 0.268$, df = 1, p = 0.605). These patterns are visualised in ***Figure 4***.

***Figure 4:** Variation in levels of female resistance to male copulation attempts (proportion of attempts resisted during twenty-minute observation). **A)** shows proportion of attempts resisted in young birds, and **B)** shows the same in old birds. Each point represents the mean per house across all sessions where at least five copulation attempts were seen. Some houses are missing from the "Morning" panels, because some houses had no observation sessions meeting this criterion. Error bars represent standard error.*

Female resistance had a significant effect on the success rate of copulation attempts (χ^2 = 76.258, df = 3, p < 0.001). Looking separately at the two components of copulation success, female resistance had a significant effect on the first component, the proportion of attempts resulting in mounts (χ^2 = 78.710, df = 3, p < 0.001), but not on the second component, the proportion of mounts resulting in copulation (χ^2 = 6.123, df = 3, p = 0.106).

No interaction effect was detected between female resistance and time of day, either for the first (χ^2 = 2.737, df = 1, p = 0.098) or second (χ^2 = 0.443, df = 1, p = 0.506) component of success, or the overall success rate (χ^2 = 0.179, df = 1, p = 0.673). Similarly, no interaction effect was detected between female resistance and age, either for the first (χ^2 = 2.425, df = 1, p = 0.119) or second (χ^2 = 0.140, df = 1, p = 0.709) component of success, or the overall success rate (χ^2 = 0.830, df = 1, p = 0.362). These relationships are visualised in ***Figure 5***.

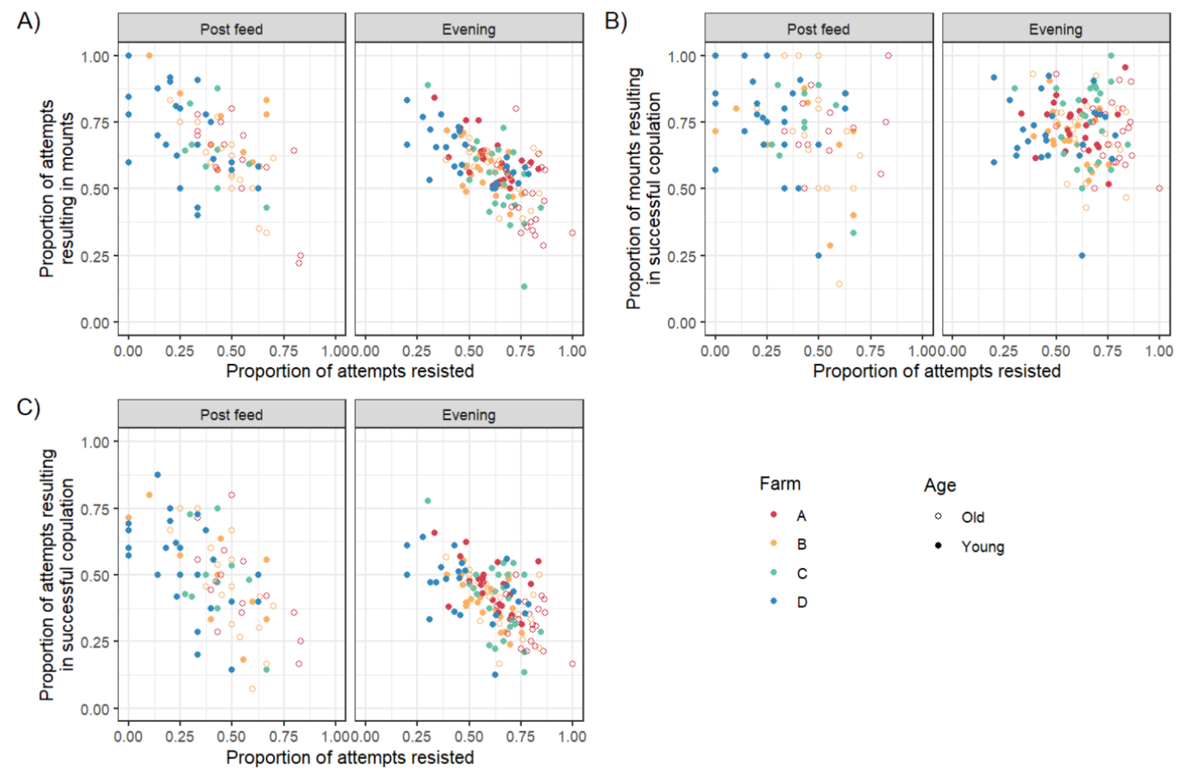

***Figure 5:** Relationship between female resistance and different components of copulation success. **A)** shows the proportion of copulation attempts resulting in mounts (the first component of copulation success). **B)** shows the proportion of mounts resulting in copulation (the second component of copulation success). **C)** shows the proportion of copulation attempts resulting in copulation (the proportion of attempts that are successful in both the first and second component). Each point represents one observation session where at least five copulation attempts by males were observed.*

v) Variance in fertility and its relationship with copulation rate

Eggs laid by "old" females had fewer sperm hydrolysis points than eggs laid by the females of the same house when they were "young". Zero counts were significantly more likely in "old" eggs ($\chi^2 = 17.398$, df = 1, $p < 0.001$), and the magnitude of values that were not zero was significantly lower ($\chi^2 = 16.101$, df = 1, $p < 0.001$). However, this effect was not moderated by which house the eggs came from (no significant effect of adding a random intercept for house to both the main model structure and the zero-inflation term: $\chi^2 = 0$, df = 1, p = 1.000), indicating that this age-dependent pattern was similar across flocks. These patterns can be seen in ***Figure 6***.

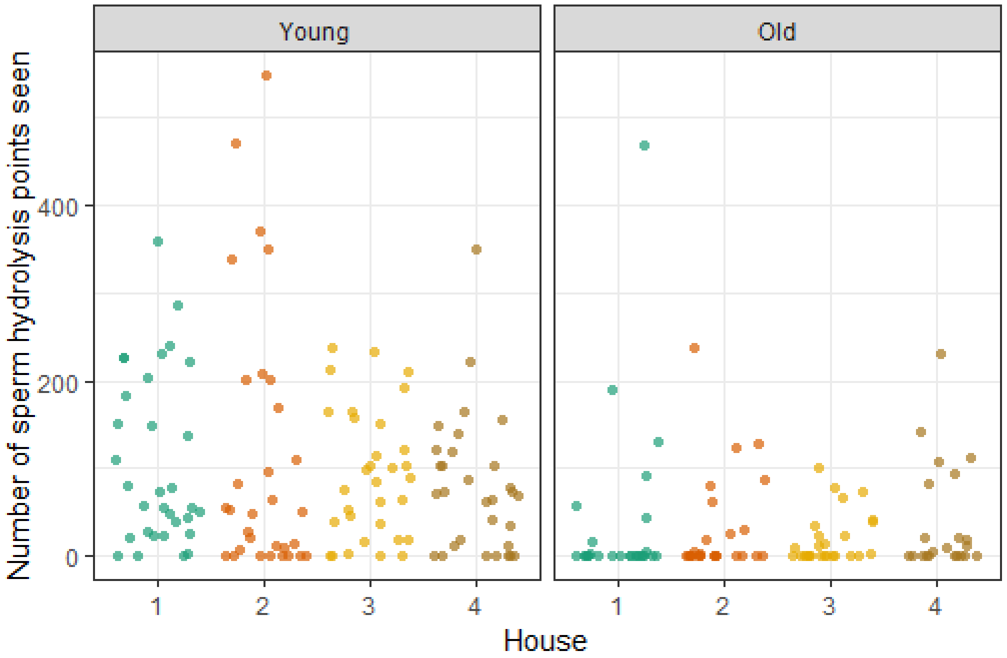

Figure 6: *Result of fertility analysis via count of sperm hydrolysis points seen in perivitelline layer of eggs from four different houses on one farm at two different time points. Each point on the graph represents one egg, coloured according to which house it came from.*

Similarly, the probability of observing a germinal disc with a fertile appearance to the naked eye was significantly predicted by age ($\chi^2 = 22.448$, df = 1, $p < 0.001$). Again, this trend was not significantly affected by which house the egg came from (no significant effect of specifying random intercept for house: $\chi^2 = 0$, df = 3, p = 1). This trend can be seen in ***Figure 7***.

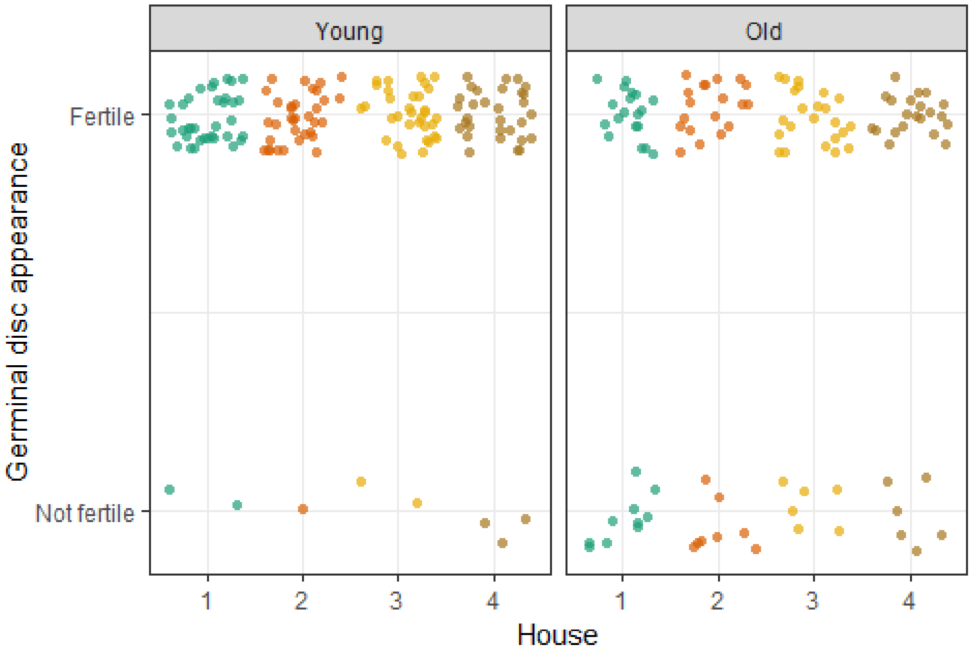

Figure 7: *Germinal disc appearance categorised as fertile or not fertile, for eggs from four different houses on one farm at two different time points. Each point on the graph represents a single egg, coloured according to which house it came from.*

Overall, the number of sperm hydrolysis points did not predict the fertile appearance of the germinal disc (no significant difference between model in which age predicts fertile appearance, with a random intercept for house, and an alternative model with an additional fixed effect for number of hydrolysis points: $\chi^2 = 1.717$, df = 1, p = 0.190). However, if we model fit these same models separately for the young and old timepoints to account for different distributions in each, then we find that the number of hydrolysis points does predict fertile appearance of the germinal disc in “old” ($\chi^2 = 15.495$, df = 1, $p < 0.001$), but not “young” eggs ($\chi^2 = 0.683$, df = 1, p = 0.409). This relationship is plotted in ***Figure 8***.

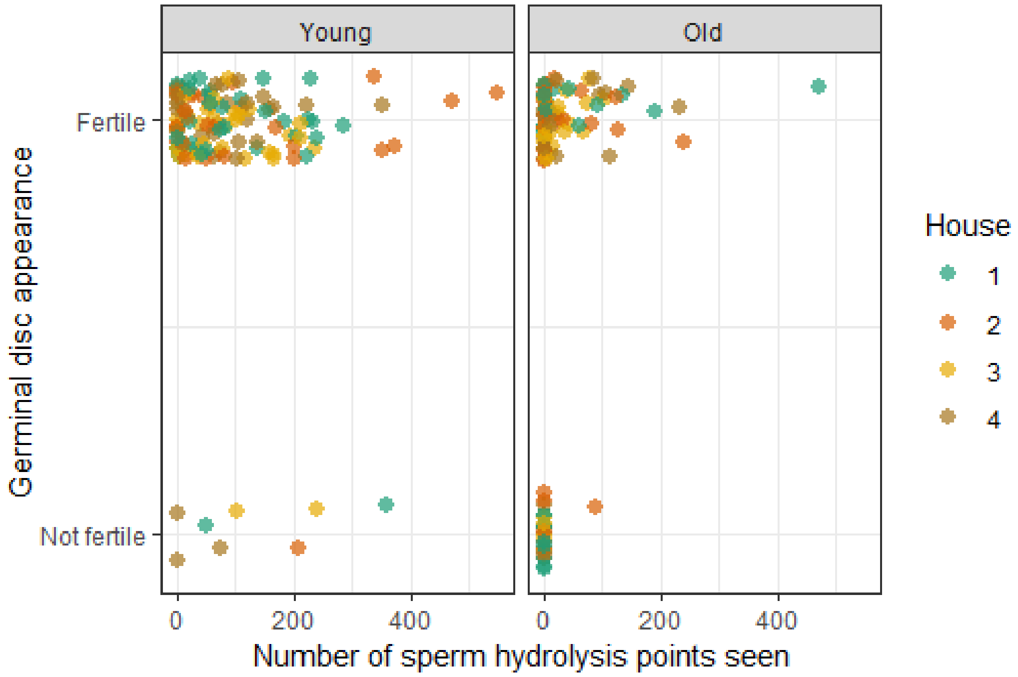

Figure 8: *Relationship between number of sperm hydrolysis points seen on the perivitelline layer and the appearance of the germinal disc (fertile vs not fertile). Each point on the graph represents one egg, laid in one of four houses on one farm at one of two time points, young or old.*

No relationship was detected between the mean copulation frequency observed in the evening for houses at Farm D at the "young" time point and either measure of fertility at either the "young" or "old" time point (95% confidence interval for Pearson's correlation coefficient: **PVL, young:** -0.97, 0.94; **PVL, old:** -0.51, 1.00; **GD, young:** -0.98, 0.93; **GD, old:** -0.97, 0.94; ***Figure 9***).

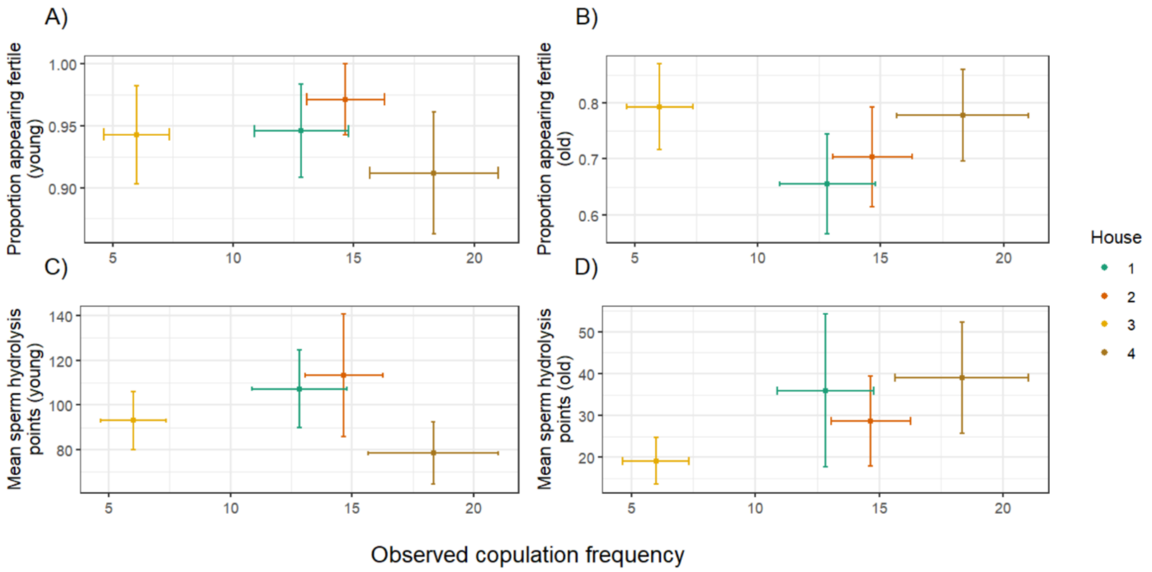

***Figure 9:** Relationship between fertility and observed copulation rate. **A)** and **B)** show the proportion of germinal discs with fertile appearance at the "young" and "old" time points, respectively. **C)** and **D)** show the mean number of sperm hydrolysis points observed per egg at the "young" and "old" time points, respectively. The y axis of all graphs shows the mean number of copulations observed during evening observations in young birds. Each point represents the mean value for one house, all on the same farm. Bars represent standard errors. No relationships were detected between copulation rate and any of the four measures depicted (see main text).*

Discussion

One of the aims of this study was to characterise variance in copulation rate within and across commercial broiler breeder flocks, and determine which factors explain this variance. Our results reveal time of day as the most important explanatory variable, with a marked circadian pattern in copulation rate. The fact that a strong peak in copulation rate was seen in the evening is consistent with the findings of previous studies of domestic chicken and feral fowl populations (Bilcik and Estevez 2005; Løvlie and Pizzari 2007; Moyle et al. 2010). The secondary peak seen in post-feed sessions appears to be a property of the broiler breeder system. A significant decrease in female resistance was also seen in the post-feed sessions, suggesting that this peak in mating activity is a product of both male and female motivation to mate at this time.

The increase in copulation immediately after feeding would suggest that optimal timing of feeding would have the potential to have a large influence on flock fertility, since the probability that an insemination results in fertilisation varies strongly with time of day (Bakst, Wishart, and Brillard 1994; Lake and Wood-Gush 1956). It also raises the question of whether splitting the daily feed allocation into more than one mealtime might have a beneficial effect in increasing the overall rate of copulation. Similarly, the “skip a day” feeding system that was used for some time in American farms (where two days’ food would be given at once (McDaniel, Brake, and Bushong 1981)) may have a detrimental effect on the rate of copulation and thus fertility.

Another important explanatory variable was the age of the birds of a flock. We observed a decline in copulation rate as birds age, driven by a decline in the rate of copulations seen in the evening sessions: for old birds, the copulation rate in the evening is approximately half of what it was for young birds. This result is expected, as senescence-related drops in copulation rate are known in this species (Duncan, Hocking, and Seawright 1990; Hocking and Bernard 1997). McGary, Estevez, and Russek-Cohen (2003) show that many other reproductive behaviours steeply decline with age, including courtship, aggression, forced copulations, and mounts. However, the causes of declines in copulation rate are not known. One explanatory factor may be that age-related weight gain and increasing prevalence of musculoskeletal problems may restrict mobility and make copulation more difficult for birds of both sexes (Hocking and Bernard 1997). In addition, decreasing libido with age is widely observed across animal species, so it may be that ageing broiler breeders are simply less motivated to mate (Bribiescas 2006; Brito et al. 2020; Grotewiel et al. 2005). Ordas et al. (2015) find that administering small doses of testosterone to ageing broiler breeder males revives hatchability to a similar degree to “spiking” flocks with new, young males, suggesting that male motivation to copulate may be key.

This decline in copulation rate is mirrored by declines in fertility in older birds seen via two different methods of assessment, confirming existing literature suggesting that behavioural change may be in part responsible for this decline. No relationship was detected between copulation rate and fertility per house, however, the small sample size for this question means that detecting a relationship was unlikely. The finding that lower fertility is characterised by both a higher proportion of eggs with no hydrolysis points detected and a smaller mean number of hydrolysis points for those eggs with a non-zero count is consistent with previous findings in broiler breeder flocks (Hazary, Staines, and Wishart 2000), and lends support to the idea that multiple processes contribute to fertility senescence.

Duncan, Hocking and Seawright (1990) report that copulation rates in singly-housed broiler breeder males declined with age, but that these declines alone were not sufficient to explain declines in fertility. Other factors that might contribute to declines in fertility include age-related declines in ejaculate quality (Dean et al. 2010), and the fact that older females are less efficient at storing sperm, meaning more copulations may be needed to achieve the same fertility (Bramwell et al. 1996; Brillard 2003). This is supported by Bramwell *et al.* (1996), who report that when artificially inseminating broiler breeders with a constant volume of semen, female age is more significant than male age in dictating the number of sperm hydrolysis points found in the perivitelline layer. However, Brillard and McDaniel (1986) find that fertility senescence in a dwarf strain of broiler breeders could be remedied by weekly artificial insemination of hens with a large quantity of sperm. This suggests that a major contributory factor is the transfer of sufficient sperm from males to females.

Age is a key modulator of competitive mating success and reproductive success in fowl. Older male junglefowl are less successful at securing mating opportunities, and have lower paternity shares post-mating (Carleial et al. 2020; McDonald et al. 2017). However, the declines in mating success and reproductive success in the broiler breeder flocks studied cannot be attributed to decreased competitive ability of older males, since all males in these flocks are the same age. This suggests that senescence in copulation rate and fertility has a strong intrinsic component.

For poultry meat production, age-related declines in fertility represent a systematic loss of productivity on a large scale. The poultry industry has tried several ways to mitigate this, such as routine artificial insemination in older flocks and “spiking” (adding new males in older flocks to boost copulation rates), with limited success (Bramwell et al. 1996). Understanding what drives declines in fertility and copulation rates could lead to the more effective deployment of interventions designed to counter it.

Another aim of this study was to determine whether there is significant variation in behaviour or fertility between farms, or between houses on the same farm. We found that copulation rate did significantly vary by both farm and house within farm. While differences between the behaviour of birds on different farms might be explained by small differences in husbandry practices, differences between houses on the same farm would suggest that there is a significant amount of stochastic variation, even between flocks within houses of the same farm, which are kept under very similar husbandry conditions. Such stochastic variation would be very unlikely in flocks of this size if the behaviour of individuals in the flock varied independently, as we would expect them to “average out” within large flocks. Instead, the presence of significant between-house variation suggests that small differences may be amplified via feedback loops (Pizzari 2016), which may give rise to emergent flock-specific properties. (Alternatively, it is also possible that some physical property of the individual houses on farms influenced the behaviour of flocks, but this is unlikely as houses were near-identical).

Looking at young and old birds separately, we found that copulation rate varied significantly by farm and house within farm for young birds, but that this effect disappeared for old birds. This might suggest that, as birds senesce and copulation rates decline, there is an erosion of variation. In addition, this finding lends support to the explanation that inter-house variability detected in young birds is truly stochastic, since it was not found to persist as birds age. Alternatively, it is possible that this difference is a statistical artefact, because while four farms (sixteen houses in total) were observed at the “young” time point, only two of these farms (eight houses in total) were observed again at the “old” time point. We did not detect any relationship between mean copulation rate per house at the two time points.

We did not detect any significant variance in fertility by house; fertility was only studied for the houses on one farm, so it was not possible to investigate differences by farm. This could indicate that physiological or post-copulatory processes may "even out" behavioural differences to result in relatively uniform group-level fertility; however, again, since behaviour was studied in more houses than fertility was, it is difficult to draw any firm conclusions.

The results of this study shed light on which behavioural traits are associated with high rates of copulation. We find a positive association between copulation rate and with the number of unsuccessful copulation attempts, the number of unsuccessful mounts, and the proportion of unsuccessful attempts that result in mounts, across all evening observation sessions. The observation that observation sessions characterised by high rates of successful copulation also had high rates of unsuccessful attempts and unsuccessful mounts suggests that gains in copulation rate are not driven primarily by a higher success rate but by a higher propensity to make attempts.

In addition, we detect a negative association between the number of copulations observed and the proportion of copulations which are resisted by females. Further investigation reveals that female resistance seems to influence mating success by preventing males from mounting, rather than by preventing copulation once mounting has occurred. Of all copulation attempts that are not successful, the majority (67%) did not result in mounts. Only 33% of unsuccessful attempts failed after mounting. Female resistance has a large impact on the probability that males fail to mount (the first component of copulation success). However, no effect was detected on the probability that mounts lead to copulation (the second component of copulation success).

This suggests that once males achieve mounts, they are likely to achieve copulation and that females are unlikely to prevent copulation from occurring at this point (for example, by uttering distress calls that promote intervention by other males (Løvlie, Zidar, and Berneheim 2014)). Instead, copulation attempts that fail after mounting may fail due to male factors, such as a lack of agility. The fact that a significant effect was seen of female resistance on the probability that attempts result in mounting suggests that males find it difficult to achieve mounts without female cooperation, which may be the reason for the larger proportion of attempts which fail at this stage.

No interaction effect was detected between female resistance and age or time of day, for either the overall success rate of copulation attempts or either of the two components of success. This indicates that the influence of female resistance on copulation success does not substantially change diurnally or over the course of the lifetime of these birds. In addition, no significant difference in female resistance was detected between young and old birds. Despite this, a lower number of copulations was seen in old birds than young birds, contrary to some findings in junglefowl suggesting that older females are more resistant to mating (McDonald et al. 2019). This suggests that declining copulation rates in broiler breeders are not caused by decreased female receptivity. It also suggests that female resistance does not occur primarily as a means of reducing copulation rate (or we might expect to see decreasing resistance in line with decreasing copulation rate as birds age). This might indicate that the main purpose of resistance is as a means of mate choice.

Collectively, these results suggest that both male and female mating motivation contribute to determining the copulation rate: the number of copulations is strongly predicted both by the number of attempts made by males, the number of mounts achieved by males, and the level of female resistance, which predicts the conversion of attempts to mounts. Mating motivation in both sexes of this species is very heavily influenced by the social environment (Johnsen, Zuk and Fessler, 2001; Pizzari *et al.*, 2003; Cornwallis and Birkhead, 2006). Therefore, establishing which features of the social environment mediate mating motivation in the specific context of broiler breeders is likely crucial for achieving higher fertility. This might be used to target effective interventions to increase fertility. For instance, if sexual novelty promotes higher mating motivation in either sex, then measures such as "spiking" (the addition of new, younger males to old flocks) or means of enrichment that promote flock mixing (Leone and Estévez 2008) might be beneficial. On the other hand, if female receptivity declines in response to increased harassment, then increasing male mating motivation may prove counterproductive. The finding that female resistance is decreased in the post-feed period suggests that manipulations of the feeding regime would be a valuable avenue for future research.

Although female resistance seems to be important, we do not find any association between the number of copulations and the number of solicitations by females. Since solicitations are under full female control, they can be thought to represent "pure" female choice, whereas female resistance or acceptance of attempts by males represents a combination of male and female choice and may be influenced by processes such as convenience polyandry or compensatory resistance. A finding that solicitations had a significant effect on the number of copulations would be a sign that proactive female choice was an important feature of the sexual dynamics of these birds: we did not find this. However, since solicitations are a much rarer event than male-initiated copulation attempts, detecting a statistically significant relationship was always going to be unlikely. The proportion of attempts initiated by females was similar in this study to that observed in junglefowl in chapter 2: slightly under 5% of mating interactions in both cases.

We did not find any association between copulation rate and the rate of courtship, or between copulation rate and intra-sexual aggression in either sex. Notably, these behaviours were very rarely observed in comparison to copulations or copulation attempts, with a majority of observation sessions having a zero count for any one of these variables. Not only does this mean that any link is statistically more difficult to detect, but it also suggests that there is little room for houses to vary in these measures and that differences in these measures are unlikely to be of much biological significance. The rarity of aggression confirms previous findings of increased social tolerance as dominance hierarchies break down in large groups (D'Eath and Keeling 2003; Estevez, Keeling, and Newberry 2003; Estevez, Newberry, and De Reyna 1997), and together with the rarity of courtship behaviour, perhaps reflects generally low levels of activity.

The relationship between courtship and copulation may be different for broiler breeders than for junglefowl and for lines of domestic chickens which have not undergone rapid artificial selection. (Millman, Duncan, and Widowski 2000) report very low rates of courtship and high rates of forced copulation and aggression from males to females. Contrary to expectations, (McGary, Estevez, and Russek-Cohen 2003) report a *positive*, rather than negative, relationship between courtship displays and the rate of forced copulations in broiler breeders. It is possible that low levels of activity in broiler breeders and the unnatural pattern of selection mean that most courtship behaviour has been “lost”, along with the scope for courtship to influence copulation in the normal way.

The rarity of courtship and of aggression (which mediates social hierarchies) raises questions of on what basis mate choice operates. If these behaviours do not occur at a high enough frequency for females to accurately distinguish between high quality and low quality males, this could have negative implications for the quality of offspring. It might be that females rely on other signals of male quality, such as ornament size, or alternatively it might be that females are less selective. Because our data is on a group level, it is not clear how selective females are with mating partners: we know that their receptivity is a determinant of copulation rate, but it might be that they resist attempts as a means to lower copulation rate rather than as a means of mate choice. Alternatively, selective resistance might allow females to select for mate novelty *per se* without necessarily being sensitive to any qualities of the male.

This study provides a rare insight into patterns of variation in socio-sexual behaviours within and across commercial broiler breeder flocks. Studies of broiler breeder sexual behaviour in a commercially realistic setting are rare: we have shown that some patterns seen in smaller groups (a peak in copulation rate in the evening, and declining copulation rate with age) are replicated in large commercial flocks. We also find that group-level behavioural measures can significantly vary between these large commercial flocks, even for flocks living on the same farm at the same time. In addition, we find that the period immediately post-feeding shows unique behavioural characteristics, with a secondary peak in copulation (smaller than the evening peak) occurring at this time, and a lowering of female resistance to mating attempts. Female resistance to mating attempts is shown to predict both the number of successful copulations and the success rate of copulation attempts on a group scale. These findings have the potential to inform husbandry practices for more effective management of welfare and fertility in these flocks.

Discussion

"Confronted with a population of males competing among themselves, often with adverse consequences for females and their offspring, one would expect natural selection to favor those females most inclined and best able to protect their interests"

- Sarah Blaffer Hrdy, *American Scientist* 1977 65(1).

This thesis studies determinants of copulation rates from the point of view of females (**Chapter One** and **Chapter Two**), of males (**Chapter Three**), and then of groups comprising both females and males (**Chapter Four**).

I begin by exploring female influences on copulation frequency, via a theoretical model (**Chapter One**) and then via an experiment on red junglefowl (**Chapter Two**). Moving on to commercially relevant broiler breeders in **Chapter Three** and **Chapter Four**, I expand the scope to include fertility as well as copulation. The link between behaviour and fertility on an individual level is examined in some detail in chapter three, followed by observations of group-level behaviour with a view to improving group-level fertility in **Chapter Four**.

The main contribution of this work is an enhanced understanding of female responses to sexual coercion, both in this species and in general: the types of response available, their effectiveness, and when we expect to see them deployed. In addition, the results of this thesis collectively illustrate the importance of chance in determining the outcomes of sexual interactions. Finally, this work also has considerable applied ramifications, and future directions for the management of broiler breeders are illuminated.

In the following sections, I discuss the main findings of this thesis in each of these domains.

Sexual conflict over copulation, and female responses to it

Conflict between individual females and males over copulation, and female strategies in response to that conflict, is a theme that runs through all chapters of this thesis.

In **Chapter One**, I explore, using a theoretical model, the optimal response of a hypothetical female of any species to conflict over copulation, and how this is expected to vary given different circumstances of ecology and life history. I demonstrate that females ought, in some situations, to begin resistance before their optimum number of copulations has occurred: this is termed "compensatory resistance".

In **Chapter Two**, I investigate different means by which female red junglefowl control copulation rate within a setting of intense conflict over copulation. Females may solicit copulations, and they may either accept or resist attempts at both forced and unforced copulation by males. In addition, I provide for the first time compelling quantitative evidence suggesting that females of this species use perching as a means to control copulation rate, and that they do this despite it being a costly form of resistance. When familiar males are removed and replaced with novel males as part of the experiment in this chapter, the level of sexual conflict over copulation increases and females are faced with a greater intensity of sexual harassment. Nevertheless, observed patterns of copulation frequency suggest that males do not “win” this sexual conflict over copulation, and that females are able to influence the outcome of sexual interactions.

In **Chapter Three** and **Chapter Four**, I present data on female resistance to copulation attempts in broiler breeders. In **Chapter Four**, I find that the proportion of copulation attempts which females resist is negatively associated with the number of copulations observed and the success rate of copulation attempts. Exploring further, I find that the effectiveness of resistance appears to be in preventing attempts from resulting in mounts: most mounts result in copulation, and resistance is not associated with the conversion of mounts to copulations. There did not seem to be any difference in the effectiveness of resistance depending on the time of day or on the age of birds. In addition, I find that female resistance is lower during the small peak in sexual activity after birds are fed than during the large peak in the evening.

Conflict over copulation occurs not just because males and females differ in their optimum copulation frequency (Arnqvist and Nilsson 2000), but also in their level of choosiness (Trivers 1972), and their optimum timing (Maklakov, Kremer, and Arnqvist 2007) and duration (Friesen et al. 2013; Thornhill and Sauer 1991) of copulation. Females are often at a disadvantage in conflict over copulation, for several reasons (Chapman et al. 2003). Selection on males to obtain more copulations may be stronger than selection on females to avoid any given copulation (Winkler et al. 2021). As a consequence of selection resulting from male intrasexual competition, males in many species are more aggressive than females, an aggression which can also serve the purpose of punishment and intimidation of females in intersexual conflict (Clutton-Brock and Parker 1995; Hrdy 1981; B. Smuts 1992; B. B. Smuts and Smuts 1993; M. Wilson and Daly 1985; Wrangham and Peterson 1996).

Females may be constrained in their ability to evolve counteradaptations by the adaptations needed for parental care. For instance, while the main purpose of a penis is to transfer sperm, the vagina must serve the dual purposes of receiving sperm and ejecting eggs or live young, which may constrain female ability to engage in antagonistic genitalic coevolution (Brennan and Prum 2015); the fact that several orders have evolved separate openings for copulation and the bearing of young might suggest that this constraint has a significant negative effect on female fitness (Eberhard 1985). In addition, female ability to evolve counteradaptations may be constrained by “sexy sons” effects. By definition, if a new adaptation gives a female a marginally increased ability to resist males compared to other females, then the males she differentially resists will be those with the best ability to overcome the resistance of the existing population of females. All else being equal, therefore, she is expected to have sons who inherit less ability to overcome female resistance than the sons of other females without this adaptation (Eberhard 1985). Female resistance adaptations must therefore have a large enough benefit to outweigh this cost if they are to evolve.

Disadvantage may also in some cases be simply baked into the structure of conflict. Regarding infanticide in langurs as a male strategy, Sarah Blaffer Hrdy points out that “pitted against a male who has the option to try again and again until he finally succeeds, females have poor odds” in defending their infants (Hrdy 1977). Or, as the Irish Republican Army famously said following a failed assassination attempt against members of Margaret Thatcher’s cabinet in 1984: “Today we were unlucky, but remember we only have to be lucky once. You will have to be lucky always” (Taylor, 2001, citation via Wikipedia). The same may be true for forced copulations. This is alluded to by Rowe (1992), who points out that in a population with a strongly male-biased sex ratio, a female who successfully repels one harasser may find that this simply enables another to take his place. In such situations, the chance of a female achieving the optimal outcome (not copulating) is slim: she would need to be lucky every time.

The costs imposed on females by sexual conflict can be strong (Rankin, Dieckmann, and Kokko 2011), and females are expected to be under selection to reduce these costs where possible. Even where sexual conflict cannot be “won” (i.e. a female achieves her optimum outcome), a female may have a selective advantage over her competitors if she is able to move the outcome of interactions closer to her own optimum, rather than simply giving up and accepting the outcome imposed by males. In the face of these stacked odds, female strategies in response to sexual coercion (including forced copulations) may be subtle and ingenious. These strategies often involve “making the best of a bad job”, anticipating and mitigating expected losses to sexual conflict.

An example of a female strategy meeting this description would the evolution of labyrinthine genitalia that impede fertilisation (Brennan et al. 2007; Orbach et al. 2017), and, to a certain extent, cryptic female choice more broadly: these could be understood- excuse the teleological shorthand- as evolution “accepting” that forced copulations will occur, and “planning” accordingly.

Another example would be convenience polyandry, in which females accept copulations from multiple males, not because such copulations are beneficial but because they are less costly than resisting them would be. Convenience polyandry occurs when the optimal outcome (not copulating) is inaccessible due to male coercion, so females pre-emptively select the next best outcome (copulating, but avoiding costly resistance). In **Chapter One**, I describe a mathematical model that predicts when convenience polyandry is expected, as defined by the cost and benefits of copulation, the cost of resistance, the effectiveness of resistance, and the number of copulation attempts expected.

Compensatory resistance, a concept introduced and developed in **Chapter One**, is another example of a female strategy mitigating the negative effects of sexual coercion. Compensatory resistance can be thought of as a complement to convenience polyandry, because while convenience polyandry is a kind of “false acceptance” of copulation attempts (accepting even though the optimal outcome is not to copulate), compensatory resistance is similarly a “false resistance”, resisting even though the optimal outcome is not for all resisted copulations to be prevented. While this remains purely theoretical at this point, if females were found to actually deploy this strategy it would be an example of an adaptive behaviour “recognising” that forced copulations will inevitably occur, and making the best of a bad job.

In this thesis, I demonstrate the importance of a detailed consideration of female resistance to copulation attempts: that the same proximate behaviour could be interpreted in multiple ways as to its ultimate evolutionary motivation; that multiple strategies are available for females to control whether copulation occurs, each with different costs and benefits; and that female resistance is relevant to the poultry industry as it is negatively associated with copulation rate.

The importance of female factors in fowl has been known for a long time. However, it has usually been treated in quite a blunt way, for instance, describing resistance using a single scale rather than as multiple separate behaviours, or assuming that females resist only because a certain copulation is not in their interest. For example, Løvlie, Cornwallis, and Pizzari (2005) describe the level of resistance to mating in female red junglefowl using a scale ranging from 1 (female solicits mating by crouching) to 6 (male grabs and copulates with female, who resists the entire copulation). A similar scale ranging from 1 to 5 is used in another paper by two of the same authors (Løvlie and Pizzari 2007). However, this is a scale that describes interactions of male and female behaviour, and so the scores of females will be constrained by the behaviour of males. For instance, if a female is not subjected to any attempts at forced copulation, she cannot score higher than 3 out of 6. This is especially problematic for studies such as the one in **Chapter Two**, where we want to control for the influence of male behaviour, which is expected to vary systematically over the course of the experiment. These scales are more a measure of the effort expended by females in resisting mating attempts (which as we have seen in **Chapter One** is a product of not only their motivation to avoid copulation but also several other factors) than a measure of their underlying propensity to copulate.

A more accurate view of females’ copulation propensity- and whether, for instance, this is shaped by costs and benefits per instance or per partner- can be gained by studying separately each means females have to control copulation. **Chapter Two** of my thesis is the first research that I have come across in this species to give female sexual behaviour such a detailed treatment; that such a treatment is warranted is, I believe, borne out by the rest of the thesis.

Often, resistance is viewed through the lens of male, rather female, fitness. In an influential early paper on sexual conflict over copulation, Rowe (1992) in passing characterises copulation attempts which females resist as “successful struggles” if copulation ensues, and “unsuccessful struggles” if copulation is prevented. This is a clear indication of which sex’s perspective is assumed to be the default (Hrdy 1986). I hope this thesis illustrates that insight can be gained by studying resistance to copulation as a female strategy in its own right, rather than simply as an obstacle to male copulation success.

The role of chance

Another contribution of this thesis is in illustrating the role of chance in determining outcomes, on both an individual and a group level.

In **Chapter Three**, I demonstrate statistically that chance- including effects caused by individual females, which are experimentally allocated to a male by chance- could explain a large proportion of variance in mating success and reproductive success for broiler breeder males in the single sire system. This is despite the males living in almost identical environments to one another, and there being no interactions with other males which would introduce more scope for chance variation.

In **Chapter Four**, I find that flock identity is a significant factor in explaining variation in copulation rate on a flock level. This is attributed to chance differences, since flocks on the same farm at the same time have almost identical environments. This suggests that flocks are complex systems in which emergent, group-level differences in behaviour can arise from self-reinforcing cycles in which one individual's behaviour affects others (Tommaso Pizzari 2016; Rickles, Hawe, and Shiell 2007). This means that small differences between individuals could be multiplied to give large differences on a group level. On the other hand, if, instead, each individual's behaviour were independent from the behaviour of others, then we would expect chance differences between individuals to "average out" over the very large groups studied, resulting in uniformity or near-uniformity between flocks.

But what does it mean to say that chance influences outcomes? If we accept that we live in a deterministic world, then (with the exception of quantum effects on subatomic processes) every event has a cause: "chance is just a word expressing ignorance" as to what that cause is (R. Dawkins 1976). Chance, in the context of this thesis, essentially means small, unknown causes that are pooled together into the error term of models.

An example I gave in the introduction of **Chapter Three** for an effect of chance was a male's encounter rate with other individuals, excluding effects of his own stable phenotype such as his activity level. However, individuals don't encounter each other *for no reason*; they each started at opposite sides of the pen, say, and walked in directions that led to their crossing paths because stimuli caused their nervous systems to direct their legs to walk at a certain pace, *et cetera*. In theory, it ought to be possible for a supremely advanced model, containing omniscient knowledge about the inner state of each bird and every minute environmental detail, to make accurate predictions of the movements of all the birds in the pen, which would leave nothing about the encounters between birds to be described as "chance".

What, then, is the significance of chance? Chance, in this context, does not mean a magical process in which events just happen for no reason. But it could be thought to mean "it might as well be no reason, for our purposes today"; chance differences are those which are not associated with any variable of interest. When we say that one male "randomly" achieves higher reproductive success than another, the randomness refers to causes which we aren't interested in: in this case, differences cannot be attributed to the identity of males; it was not the males themselves that caused the differences. In the case of estimating opportunity for selection, as in **Chapter Three**, variance in reproductive success that is attributable to chance is not variance on which selection has the potential to act, so estimating how much variance is explained in this way is useful for estimating the "true" opportunity for selection.

Many authors have written on the importance of understanding the distributions that would be expected by chance in the context of variance in reproductive success. An early illustration was Sutherland's (1985) reappraisal of Bateman's (1948) classic study in *Drosophila*. While Bateman interpreted greater variance in male reproductive success to be a sign that sexual selection was operating on males, Sutherland showed that this variation is within the bounds of what might be expected according to an occurrence of copulations modelled by the Poisson distribution. Sutherland invites us to consider "two males and two females of a species in which males spend a negligible amount of time in mating and looking after the offspring. If the females mate at random, then it is as likely that one male will inseminate both females as it is that each male will mate once." An observer in this case might be tempted to assume that a male who copulates twice while his neighbour does so not at all must have a much fitter phenotype; however, there is no need to reach for this explanation. And crucially, variance in male traits is not contributing to the distribution of outcomes, then we do not have "true" opportunity for selection.

Psychological and anthropological research shows that humans tend to underestimate the amount of variation which can be produced by statistical distributions, for instance incorrectly believing that a sequence of coin flips ought to regularly switch between heads and tails, with few long, uninterrupted runs of one side in a row (Bar-Hillel and Wagenaar 1991; Kahneman and Tversky 1972). In addition, humans are biased towards explanations for phenomena that are teleological and based on the actions of conscious, causative agents over explanations that are based on unconscious, purposeless natural processes (Kelemen 1999; Kelemen and Rosset 2009; Nichols 2004; Rottman et al. 2017). Since the phenotype of individual animals (i.e. conscious, causative agents) is the variable of interest in much biological research, it may be particularly tempting to assume that differences in outcomes are down to the individuals themselves, and not random error. It is important, therefore, for researchers in biology to remind themselves that "randomness" can produce deceptively large variation in outcomes (H Klug et al. 2010; Hope Klug and Stone 2021).

Commercial relevance

The findings of this thesis provide insight into the behaviour of broiler breeders and the influences on copulation and reproductive success in a commercial environment. In **Chapter Three** I characterise the impact of intrasexual competition on the opportunity for selection in males, through mating success, through paternity share, through mate fecundity, and in total. I also examine whether any of these show an association with the behaviour of individual males, and do not detect any association. In **Chapter Four** I describe the behaviour of large flocks of broiler breeders in a commercial environment. A key finding of this chapter is that there is a peak in mating activity after the birds are fed in the morning, characterised by low levels of female resistance and high rates of copulation. This finding, in combination with the fact that copulations in this species have varying probabilities of leading to fertilisation depending on time of day (Tim R Birkhead, Cunningham, and Cheng 1996; Lake and Wood-Gush 1956), suggests that experimenting with feeding schedules could alter group fertility. In addition, the association between levels of female resistance and copulation rate suggests that both male and female copulation propensity plays a role in determining copulation rate. This is significant as most studies of broiler breeders focus on the copulation propensity of males (e.g. Duncan, Hocking and Seawright, 1990; McGary, Estevez and Bakst, 2003). **Chapter Two** suggests that females may increase receptivity in response to sexually novel males. This might indicate that using enrichment to increase circulation of birds around houses, for example, could increase the rate of encounter with novel sexual partners and thus increase female receptivity.

Future directions

This research brings up a lot of further questions that would be interesting to test. **Chapter One** provides interesting theoretical predictions- but there is no evidence that “compensatory resistance” actually occurs in any real species. In addition, the model itself could be expanded by for instance allowing male behaviour to respond to female strategies, and predicting how these interactions might play out in evolutionary time.

Experimentally varying the costs and benefits of copulation and resistance in the immediate term would likely be more tractable in terms of seeing effects on individual behaviour than varying the costs and benefits in terms of lifetime fitness. So for example, ablating genitalic spines that cause immediate harm to females (Rönn, Katvala, and Arnqvist 2007) might be a better approach than for example using *sfp*-knockout Drosophila, which would be expected to decrease the cost of copulation on female lifespan (Chapman et al. 1995) but might not cause female behaviour to be altered in the immediate term. Drosophila would in any case not be a good choice to test this model’s predictions because females have a discrete switch, mediated by male ejaculates, between unmated and receptive and mated and resistant states (Hopkins and Perry 2022). Junglefowl, on the other hand, could be a good species to test some of the predictions of this model, due to their multiple mating and variable levels of female resistance.

Chapter Two concludes that females may benefit by prioritising novel mates, and that polyandry might be motivated by benefits to females, not just the avoidance of resistance costs. However, these conclusions are tentative, because we are limited only to data on copulations. Data on reproductive success- for instance, if we incubated and tested the parentage of eggs laid by females immediately following the experiment- would be a very useful complement to this chapter, and could help confirm or reject the conclusions reached.

Chapter Three provides insight into how opportunity for selection is partitioned for males with and without intrasexual competition. Further valuable insight could be gained by reanalysis of the same data to investigate effects on the opportunity for selection in females. Is there any evidence that male intrasexual competition depresses female fitness compared to the single male treatment? Or on the other hand, does the increased potential for mate choice and polyandry increase female fitness in the competition treatment? In order to isolate different effects that could be in play, we could manipulate the sex ratio in both treatments. For instance, it could be that female fitness is curtailed (and variation between females increased) in the single-male treatment not due to constrained mate choice but due to sperm limitation, as a result of sharing one male with eleven other females.

The conclusions reached in this chapter on the difference in opportunity for selection via different components of reproductive success with and without male intrasexual competition are somewhat tentative, due to the fact that only a small number of groups were tested, and there was significant difference between groups on the same treatment. It would be very interesting to test this on a larger scale, using many more groups per treatment, in a species like *Drosophila*. This would provide more reliable detail on the differences in opportunity for selection, as well as the level of variation seen across groups on the same treatment.

As discussed, **Chapter Four** suggests several avenues of future research that may be fruitful in optimising the management of broiler breeders. I would be interested in particular to see the results of experimenting with different feed schedules, and perhaps splitting the daily feed ration into multiple meals to see whether multiple peaks in copulation rate could be elicited. In addition, we find that female receptivity is associated with copulation rate; since most studies into broiler breeders focus on male factors, this result suggests that approaches concerning female factors may currently be under-investigated.

Conclusion

Charles Darwin's insight– evolution by natural selection– is an extremely powerful idea: the "single best idea anyone ever had" (Dennett 1995). It is powerful in that it explains a huge amount via a few relatively simple observations and logical inferences. Some reproducing entities reproduce more than others; therefore, more of this first type of entity than the second will exist in the future.

Reproduction, then, is at the heart of all evolution, not just evolution by natural selection but other forms of evolution too, such as cultural and linguistic evolution; and not just on the level of the individual but on all levels- between groups, within the soma, between sperm in a single ejaculate. As Matt Ridley put it in *The Red Queen*, "reproductive success is the examination that all […] genes must pass through if they are not to be squeezed out by natural selection” (Ridley 1993). A new mutant allele could be so brilliant in terms of survival that it made its owner immortal, but if it was not attached to alleles that allowed successful reproduction, then it would be at a total dead end in the evolutionary algorithm.

In the family tree that connects all living things, successful reproduction is at every node, and is what allows the shuffling and passing on of genes as they flow through time, from the origin of life four billion years ago to today. Viewed this way, reproduction is the single most relevant moment in any organism’s life. Sexual reproduction is an incredibly high-stakes moment of forced cooperation which motivates the formation of uneasy alliances and the evolution of all sorts of dastardly, exploitative and ingenious adaptations.

Why do some individuals reproduce, while others do not? Why, and how, do some reproduce more than others? These, to me, are the questions at the heart of biology, and are the questions that motivate this thesis.

Appendix to Chapter 1

i) Optimum number of copulations

Equation **1** is a function that models the fitness payoff of a given number of copulations, N_m.

$$\mathbf{1} \quad f(N_m) = A(1 - e^{-BN_m}) - CN_m$$

This function consists of an exponential term representing the diminishing returns of increasing numbers of copulations, with a constant term subtracting a cost C of each copulation (see ***Figure 1***, reproduced from main text of Chapter 1).

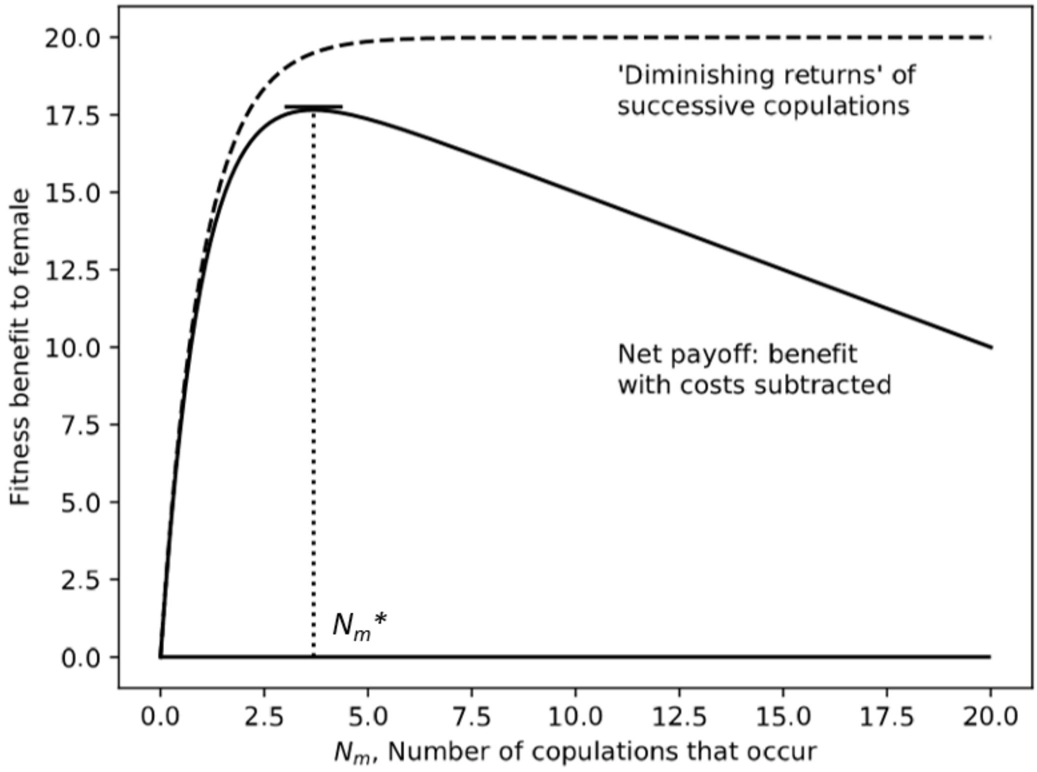

Figure 1: *The solid line shows the shape of the payoff function given in equation* ***1*** *where A = 20, B = 1, C = 0.5. Fitness increases with increasing number of copulations, N_m, before beginning to decline as the exponential term saturates and the cost term begins to dominate. The optimum number of copulations, ${N_m}^*$, is defined as the value of N_m that gives the highest payoff according to this function. The dashed line describes the case where* C = 0*, showing the saturation of the payoff of increasing N_m when there is no cost of mating.*

The optimum mating number, which we will call ${N_m}^*$, is the value of N_m that produces the greatest net benefit as defined by $f(N_m)$. At this point, $f'(N_m)$ will be equal to zero (see ***Figure 1***).

Expanding brackets and differentiating **1** gives

$$\textbf{2} \qquad f'(N_m) = ABe^{-BN_m} - C$$

We then equate this to zero and rearrange to obtain

$$\textbf{3} \qquad {N_m}^* = \frac{-1}{B}\ln\left(\frac{C}{AB}\right)$$

ii) Optimum number of copulation attempts to accept

We have produced a formula for the optimum number of copulations, but females do not have direct control over the number of copulations that occur; they can only decide the number of mating attempts that they accept. According to this model, a female receives N mating attempts and chooses a certain number N_a to accept, resisting the remainder N_r.

$$\textbf{4} \qquad N = N_a + N_r$$

Supposing resistance has a probability p of preventing copulation from occurring, we can then say that the number of copulations that occur will be equal to the number that are accepted plus the number that are unsuccessfully resisted:

$$\textbf{5} \qquad N_m = N_a + (1-p)N_r$$

If we say that resistance has a cost, r, we can say that the net payoff of any given value of N_a will be the fitness payoff (given by **1**) of the consequent number of copulations (given by **5**), with the resistance costs subtracted.

$$\textbf{6} \qquad Z = f(N_m) - rN_r$$

As in section *i)*, the optimum value of N_a, which we will call ${N_a}^*$, will occur when $\frac{dZ}{dN_a}$ is equal to zero. Using the chain rule to simplify the first term:

$$\textbf{7} \qquad \frac{dZ}{dN_a} = \frac{df}{dN_m} \times \frac{dN_m}{dN_a} - \frac{d}{dN_a}(rN_r)$$

If we write $(1-p)$ as q and N_r as $N - N_a$ (equation **4**), then equation **5** rearranges to give

$$\textbf{8} \qquad N_m = pN_a + qN$$

And so by polynomial differentiation, $\frac{dN_m}{dN_a}$ equates to p. Similarly, using equation **4** followed by polynomial differentiation, $\frac{d}{dN_a}(rN_r)$ simply gives -r. We also know from section *i)* that $\frac{df}{dN_m}$ equates to $ABe^{-BN_m} - C$ (equation **2**). Therefore:

$$\textbf{9} \qquad \frac{dZ}{dN_a} = (ABe^{-BN_m} - C) \times p + r$$

Written in terms of N_a (using equation **8**):

$$\mathbf{10} \qquad \frac{dZ}{dN_a} = p\left(ABe^{-B(\,pN_a+qN)} - C\right) + r$$

${N_a}^*$ occurs where $\frac{dZ}{dN_a} = 0$. Therefore, by substituting and rearranging **10**,

$$\mathbf{11} \qquad {N_a}^* = \frac{\frac{-1}{B}\ln(k) - qN}{p}$$

Where k is a constant defined in terms of A, B, C, r, and p:

$$\mathbf{12} \qquad k = \frac{C - \frac{r}{p}}{AB}$$

The value of k can be used to categorise the optimal female behaviour as predicted by the model into four exhaustive and mutually exclusive cases (see ***Table 1*** in main text).

i) Deriving $\mathrm{N_m}^*$ from $\mathrm{N_a}^*$

Because resistance has a cost, r, female fitness is maximised through a value of N_a which results in a number of copulations that "overshoots" ${N_m}^*$ by some amount, since the cost of excess copulations will be offset by a decrease in resistance costs (***Figure 2***, reproduced from main text of Chapter 1).

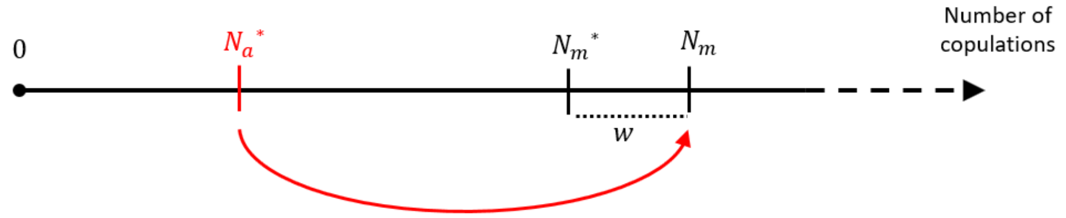

Figure 2: *Number of copulations is represented on a number line increasing from 0. Each value of* N_a *(number accepted) maps to a resulting value of* N_m *(number occurring), which is made up of the number accepted plus the number unsuccessfully resisted. The optimum value of* N_a*, (*${N_a}^*$*, marked in red) is one that will overshoot* ${N_m}^*$*(the optimum value of* N_m*) by an amount dependent on the cost of resisting. The amount of overshoot is labelled* w.

We can calculate the value of N_m that will result from ${N_a}^*$, using equation **11**, which gives us the value of ${N_a}^*$, and equation **8**, which tells us the number of copulations N_m that will result from any given number of acceptances N_a. By substituting **8** into **11** and simplifying, we obtain:

13 $$N_m = \frac{-\ln{(k)}}{B}$$

Figure 2 shows us that the value of N_m resulting from ${N_a}^*$ (as defined in **13**) is made up of ${N_m}^*$, plus some amount of "overshoot" which we call w. To obtain an expression for the magnitude of w we can subtract ${N_m}^*$, as defined in equation **3,** from equation **13**, which simplifies to give

14 $$w = \frac{1}{B}\ln\left(\frac{C}{C-\frac{r}{p}}\right)$$

Therefore, if we have an estimate for ${N_a}^*$, we can use this to say that ${N_m}^*$ will be equal to the N_m resulting from ${N_a}^*$ (equation **8**), minus w (equation **14**):

15 $${N_m}^* = p{N_a}^* + qN - \frac{1}{B}\ln\left(\frac{C}{C-\frac{r}{p}}\right)$$

Appendix to Chapter 3

Principal components analyses were conducted to determine whether correlated behavioural variables showed a relationship to mating success or reproductive success. The behavioural variables included for bulk and single sire males are detailed in ***Table 1***. All behavioural variables were standardised by subtracting the mean and dividing by the standard deviation specific to that mating group (Bulk 1, Bulk 2, Single Sire 1 or Single Sire 2).

Table 1: *Behavioural variables included in principal components analyses*

Behaviour	Explanation	Groups for which this variable was used
Copulation	Total number of successful copulations seen. Success determined by cloacal contact between male and female, either directly observed or inferred from tail position.	Bulk and single sire
Copulation attempt	Total number of copulation attempts seen, whether successful or unsuccessful. An attempt was counted if any of the following was observed: - Male approaches female from behind with hackles raised - Male grasps female's comb or head/neck feathers in his beak - Male places one foot on the back of a female - Male mounts the female with both feet on her back	Bulk and single sire
Success rate of copulation attempts	The proportion of attempts resulting in successful copulation. This was calculated by dividing the number of copulations by the number of attempts for each of the eight observations of each male, and then taking a mean across all observation sessions for each male	Bulk and single sire
Number of copulation partners	Number of unique partners a male was observed copulating with per observation session, averaged across sessions	Single sire only (unique IDs of females not known for bulk males)
Number of attempt partners	Number of unique partners with whom a male attempted copulation per observation session, averaged across sessions	Single sire only (unique IDs of females not known for bulk males)

Solicitations received	Number of times females crouch in front of males to invite copulation	Bulk and single sire
Courtship	An instance of courtship behaviour was counted if males made a food call (low clucking sound while scratching and pecking at the ground or picking up an item in his beak) or waltzed at a female (turned in an arc in front of her with outer wing outstretched and beating against leg)	Bulk and single sire
Crow	Distinctive loud call made by males with beak open and neck outstretched	Bulk only (did not collect for single sire)
Aggression (giving)	All instances of aggression including pecking, chasing, or lunging at another male, waltzing at him (see Courtship), or interrupting his mating attempt.	Bulk only (no aggression between males occurs in single sire)
Aggression (receiving)	As above, all instances of receiving one of these behaviours from another male.	Bulk only (no aggression between males occurs in single sire)

Two principal component analyses were then conducted, one for bulk groups and one for single sire groups, using the function princomp( ). The two largest components were extracted from both. For the bulk groups, the first two components cumulatively explained 62% of variance. For the single sire groups, the first two components cumulatively explained 82% of variance. Loading of variables on the two largest components for bulk and single sire males are shown in ***Table 2*** and ***Table 3*** respectively.

Table 2: *Loading of variables on two largest components for bulk males, colour coded to show magnitude of positive or negative loading.*

	Component 1 (41.8%)	Component 2 (20.4%)
Copulations	0.4636591	0.20403398
Copulation attempts	0.4498770	0.04069245
Success rate	0.2166205	0.48148328
Solicitations received	0.4176257	0.12608262
Courtship	0.3614494	-0.35429682
Crows	0.2459216	-0.69012632
Aggression given	0.3440600	0.23316256
Aggression received	0.2275850	-0.22983891

Table 3: *Loading of variables on two largest components for single sire males, colour coded to show magnitude of positive or negative loading.*

	Component 1 (52.8%)	Component 2 (28.9%)
Copulations	0.457183155	0.1363506
Copulation partners	0.460953736	0.1327558
Copulation attempts	0.416804383	-0.2804716
Attempt partners	0.478064653	-0.3145288
Success rate	0.233830697	0.4569019
Solicitations received	0.003847307	-0.7512705
Courtship	0.348628618	0.1141274

For both bulk and single sire groups, the largest component, explaining roughly half of variance, loads positively on every variable, and thus can be considered a measure of the overall level of activity of males. The second largest component in both groups shows a mixture of positive and negative loadings on different variables.

Next, a total of eight linear models were fit, regressing each of these four components against mean-standardised reproductive success (weekly number of chicks) and mean-standardised mating success (weekly number of genetic mating partners), with each of these variables being standardised using the group-specific mean. Models for bulk males included all males in both bulk groups, and likewise for single sire males, with separate slopes and intercepts for both groups.

These were compared to null models that explained reproductive success and mating success simply as a function of group (Group 1 or Group 2, for both bulk and single sire models). Model comparison by likelihood ratio test determined whether either component significantly predicted either outcome variable for bulk or single sire males.

For single sire males, neither component was a statistically significant predictor. Component 1 did not have a significant effect on reproductive success ($\chi^2 = 3.882$, df = 2, p = 0.144) or genetic mating success ($\chi^2 = 3.397$, df = 2, p = 0.191). Component 2 similarly did not have a significant effect on reproductive success ($\chi^2 = 1.627$, df = 2, p = 0.443) or genetic mating success ($\chi^2 = 0.803$, df = 2, p = 0.669).

For bulk males, component 1 was not a significant predictor either for reproductive success ($\chi^2 = 3.081$, df = 2, p = 0.214) or genetic mating success ($\chi^2 = 3.371$, df = 2, p = 0.185). However, component 2 did show a negative relationship with reproductive success ($\chi^2 = 9.262$, df = 2, p = 0.010) and for genetic mating success ($\chi^2 = 9.265$, df = 2, p = 0.010). This relationship can be seen in ***Figure 1***.

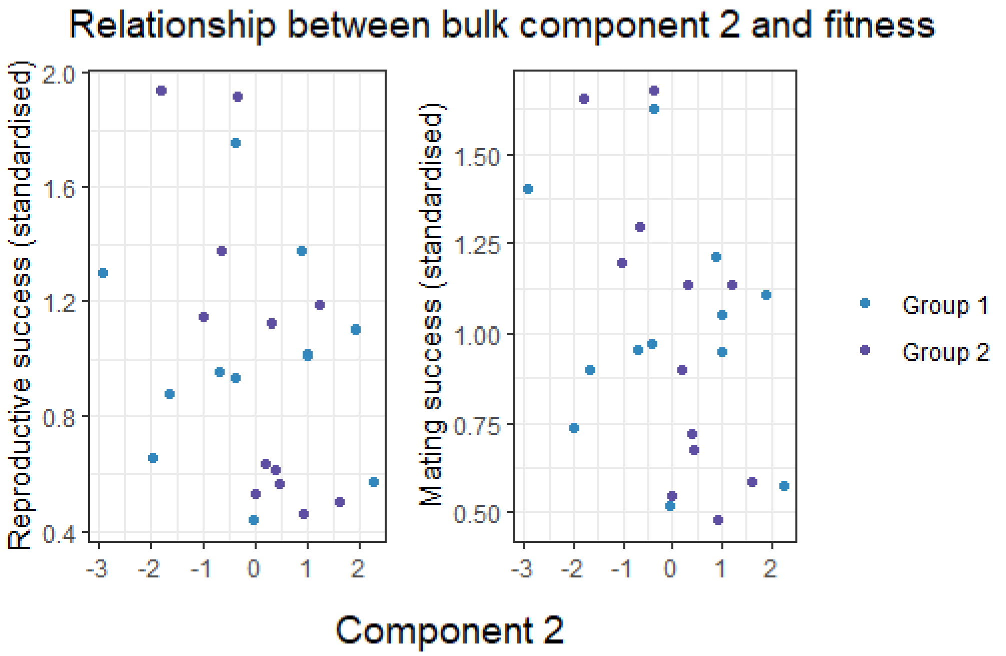

Figure 1: *Principal component 2 as a predictor of reproductive success and mating success in bulk mating groups (both standardised by subtracting mating-group-specific means). Each point on the graphs represents one male.*

This statistically significant result is unlikely to have much biological significance, however. The largest loading of any variable on component 2 is a negative loading on the number of crows. This makes sense as we expect males that crow more to have more mating success and reproductive success, and component 2 is a negative predictor of these variables. However, the component also has a positive loading on the success rate of copulation attempts, on the number of successful copulations, and on aggressive behaviours, all of which we would expect to positively, not negatively, predict mating and reproductive success.

This paradoxical result may be because, since broiler breeders show low levels of activity, general activity levels are likely to explain a large proportion of behavioural variation (as in component 1 of both bulk and single sire males). The pattern of variance in other variables left over after this is accounted for may therefore be idiosyncratic and seemingly biologically contradictory.

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With apologies for how self important this undoubtedly sounds, I would also like to acknowledge the books of Richard Dawkins, which had an enormous influence on me when I came across them in my school library over a decade ago. Without having read these books, I probably would have neither studied biology at university nor pursued a PhD in it. In particular, *The Selfish Gene* has shaped my thinking on all manner of issues and pointed me towards many areas of interest both within and outside biology, and its influence discernibly runs through this thesis, in particular the first chapter.

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