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# Mating System Of Natural Populations Of The White-footed Mouse, Peromyscus Leucopus

Xuhua Xia

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# MATING SYSTEM OF NATURAL POPULATIONS OF THE WHITE-FOOTED MOUSE, *PEROMYSCUS LEUCOPUS*

by

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Submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario
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# ABSTRACT

The mating system of the white-footed mouse, *Peromyscus leucopus* has caused much confusion among evolutionary biologists interested in the social biology of the species. Some studies documented well-developed paternal behaviour (Horner 1947; McCarty and Southwick 1977; Hartung and Dewsbury 1979) and pair-activity (Mineau and Madison 1977). These results favour a monogamous mating system. Other studies proposed polyandry based on spatial association between adult males and females (Myton 1974). Still others argued for polygyny (Cicirello and Wolff, in press). All these studies are speculative.

I studied the mating system of the species with four different approaches. I first tested whether oestrous females would reject strange males and accept familiar males as mates, then examined whether paternal behaviour documented in previous studies might be unnatural behaviour caused by confined caging conditions. Meanwhile, I investigated association patterns of adult males and females in their natural habitats, and collected genetic information on mothers and their young to test the presence of multiple paternity within single litters.

Oestrous females readily accepted multiple-male insemination, with no indication of discriminating between familiar and strange males. Males did not provide paternal care of any sort in a large enclosure with 4 nest boxes and stopped interacting with the females once the copulation was over. The majority of experimental females showed aggression towards males except during their oestrus. In their natural habitats, females close to oestrus have more adult males nearby than females far from oestrus, indicating that adult males adjusted their position in relation to breeding status of females. Genetic analysis revealed that 25-100% of field-conceived litters were sired by multiple males. I conclude that promiscuity is a prevailing feature of the mating system of *P. leucopus*, with males exhibiting serial polygyny and females simultaneous polyandry. Adaptive significance of this mating system is discussed in relation to physiology, ecology and life history characters of the species.

# ACKNOWLEDGEMENTS

First of all, I wish to thank Dr. Jack Millar, my MSc and PhD supervisor, for his patience, encouragement and information I retrieved from his magnificent head. I appreciate all he has done for me and he will always be my supervisor.

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It is Dr. Paul Handford who introduced me into the real riches of evolutionary theory and to him I am very grateful. I wish I could have known him better and earlier.

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several mistakes in different manuscripts of mine and his insights prompted me to read more and study harder.

Dr. Terence Laverty and Dr. Miles Keenleyside have both contributed to my understanding of evolutionary biology. Thanks.

I enjoyed the intellectual atmosphere in Collip Building, although the physical atmosphere is always heavily loaded with molecules manufactured continuously in the digestive and urogenital systems of animals as a revenge to human beings who deprive them of animal dignity.

Many graduate students helped me, one way or another, during my five years of stay at Western. I shared office for several years with Barb Beezley, Jackie Dixon, Jonathan Falks and Peter Hicklin, whose friendship greatly enriched my life. Joe Weaver helped cheer me up when my life was trapped in the dark valleys of financial difficulties, and he once kindly considered me as his potential business partner in selling Christmas trees. I am indebted to Roger Frapier, who is always around when I need help. Academically, I interacted most with Todd Arnold and Rich Moses, and I always envy their clear thinking and their huge reservoir of knowledge. Many former graduate students, such as Kiwi, Zoltan, A.J., Lui, Ray, Shirley, Sue, Keith, Andy and Vickie have, more or less, shaped my life for the better. I am particularly grateful to Mark Ridgeway, who is the first person in the department telling me that I would be doing alright in my study at

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# DEDICATION

This thesis is dedicated to those whose blood stained Tien-An-Men Square in June the fourth, and to their wives and mothers who wept over the permanently silenced bodies of their beloved ones.

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# CHAPTER 1 INTRODUCTION

## § 1.1 General Review

The theory of evolution by natural selection is built on heritable variation in reproductive success, of which mating success is the most important component, especially for males. Mating success of an animal in a natural population depends much on sexual and social relationships it forms with other animals, especially those of opposite sex. The pattern of these sexual and social relationships that form the context within which mating occurs is called a mating system (Halliday 1980). Mating systems are of four major categories: monogamy, polygyny, polyandry and promiscuity. A more detailed classification of mating systems can be found in Handford and Mares (1985).

Mating systems of natural populations of animal species are subject to physiological, ecological and life history constraints. The influence of life history traits on mating systems is best illustrated by studies on wild populations of the red deer, _Cervus elaphus_ (Clutton-Brock _et al._ 1982). Females in this species reach sexual maturation as yearlings. Males are capable of mating at two years of age, but do not reach full body size until they are 6 years old. As a consequence of this body size difference, sexually mature males differ greatly in their ability to compete for mating opportunities. This results in breeding females held in harems mostly by a few males from 6 to 11 years old, i.e., a polygynous mating system.

Physiological constraints on mating systems may be exemplified by modes of fertilization and parental care. Internal fertilization and specializations such as lactation predispose females to perform parental care (Williams 1975; Krebs and Davies 1987) and allow males greater opportunity to desert and seek extra matings, while external fertilization and the need for two parents to feed the young may predispose males to show parental care and necessitate stable pair-bonding relationships between males and females. In many avian species with altricial young, reproductive success is often limited by the ability of parents to feed their young (Lack 1947; Ricklefs 1977) and it is likely that two parents can feed twice as many young as a single parent. If either sex deserted, the output of the brood would be halved. So it may not pay either sex to desert if we also consider the cost in searching for a new mate and nest site for a new clutch.

As males can usually benefit more than females by seeking extra matings, any ecological factors that changes a male's opportunity of having extra matings or the ability of females to raise young without paternal care can affect the mating system and social organization (Orians 1969; Verner and Engelsen 1970; Hladik 1975; Clutton-Brock and Harvey 1977; Emlen and Oring 1977; Wrangham 1977; Ghiglieri 1984; Vehrencamp and Bradbury 1984; Terborgh and Janson 1986). For example, in a heterogenous environment, resources necessary for rearing the young are distributed in patches. If males differ much in their ability to defend territory, resulting in some males having good territories with resources more than twice as much as some poor territories, then two females sharing a good territory with the a defending male may each leave more offspring than one female pairing with a male in a poor territory. Thus a continuum of territory quality will give rise to polygyny and monogamy coexisting in the same population (Orians 1969).

Although the definition of a mating system does not extend to cover post-mating behaviour, mating systems are often studied in association with parental care. Studies on social biology of natural populations (e.g., Schaller 1965; Hall and DeVore 1965; Crook 1970; Dewsbury 1978; Mitchell 1979; Eisenberg 1981; McKenna 1982; Handford and Mares 1985; Poole 1985) have produced relationships between mating systems and parental behavioural patterns, e.g., paternal care is often found in species with a monogamous mating system, which ensures certainty of paternity, and absent in species with a promiscuous mating system. Trivers (1972) proposed certainty of paternity as a determinant of paternal care because it is obvious that caring for unrelated young is not evolutionarily advantageous.

Trivers' argument has been challenged by Maynard-Smith (1978), Werren et al. (1980) and Krebs and Davies (1987), on the following basis. The fitness gain of a non-paternal male is the chance of additional mating. But, if paternity is uncertain, the value of additional mating is reduced by exactly the same factor as the value of paternal care. Thus, whether a male should provide paternal care depends on chance of additional mating, not on certainty of paternity. I agree with them that certainty of paternity may not be an important determinant of paternal care, but empirical data in rodents demonstrate that paternal care does imply certainty of paternity (Kleiman and Malcolm 1981; Elwood 1983; Dewsbury 1985) and paternal behaviour remains a good predictor of mating system.

In summary, previous studies on mating system have formed a theoretical frame work in which predictions concerning mating systems can be made on the basis of physiological, ecological, life history and behavioural patterns and tested with field and laboratory observations.

## § 1.2 Review of Studies on Mating Systems in Small Rodents

Rodents differ much in their habits, with some being diurnal and non-secretive, such as ground squirrels, others nocturnal or crepuscular, such as most microtine rodents and peromyscine species, which I refer to as small rodents. I will briefly review those studies pertaining to small rodents except for P. leucopus, which will be dealt with in the following section.

There are few species of small rodents in which mating system has been studied in detail. The mating system of _P. polionotus_ is perhaps the best known. Burrow excavation showed that each burrow, as a general rule, was occupied by an adult male, an adult female and one litter (or two litters of different ages), and this was supplemented by a genetic study demonstrating that multiple paternity in single litters was unlikely (Foltz 1981). Thus monogamy is a prevailing feature of the mating system of the species.

Kawata's (1985) study on _Microtus_ _rufocanus_ showed that the home range of an adult male often overlaps the home range of several adult females. Presumably the male may mate with several females. Because no multiple paternity in single litters was found (Kawata 1985), each female appeared to have mated with only one male. For this reason, polygyny is inferred to be a dominant feature of the mating system of the species.

Mihok (1979) concluded that the basic social unit in _Peromyscus_ _maniculatus_ consisted a mature male, a few mature females and a number of young, based on his multiple capture data. Such a social unit implies polygyny. Birdsall and Nash (1973), however, found multiple paternity in single litters in natural populations of _P._ _maniculatus_. Thus females mate with multiple mates in the species. The mating system of the species is now believed to be promiscuity (Wolff 1989).

Of many studies on mating systems of other species of small rodents, few are conclusive. For example, promiscuity in *Microtus pennsylvanicus* reported by Madison (1980) was supported by no more evidence than the finding that the home range of sexually mature voles overlapped at least two sexually mature voles of the opposite sex. Polygyny was reported in *M. californicus* (Ostfeld 1986), *M. xanthognathus* (Wolff and Lidicker 1980), *Apodemus sylvaticus* and *Clethrionomys glareolus* (Korn 1986). All three studies base their conclusion on the finding that home ranges of adult males do not overlap and may encompass the home range of several females, and that home ranges of females overlap. Getz *et al.* (1981) reported monogamy in *M. ochrogaster*. The only field evidence for their conclusion is that about 13% of multiple captures involves a male and a female.

More confusion arises when investigators carelessly cite equivocal conclusions of other workers. For example, the study on *Microtus pinetorum* (FitzGerald and Madison 1983) was cited as evidence for polyandry by Cockburn (1988), but cited as evidence for promiscuity by Wolff (1985b), while the authors themselves actually favour monogamy. Viitala's (1984) study on *M. rutilus* was cited by Cockburn (1988) as evidence for polygyny. In fact, Viitala (1984) neither mentioned polygyny nor provided any evidence in favour of a polygynous mating system. The study by Madison (1980) on *M. pennsylvanicus* was cited by Wolff (1985b) as evidence of promiscuity but by Cockburn (1988) as evidence for polygyny. Wolff (1985c) was cited by Wolff (1989) as presenting spacing data suggesting a polyandrous mating system in Peromyscus leucopus and P. maniculatus, but the only data relevant to mating system of these two species in Wolff (1985c) are that males had larger home range than females, which does not suggest a polyandrous mating system.

In summary, mating systems of small rodents are poorly studied and speculation prevails in published works. Because mating system is known for only a few species of small rodents, it is not even possible to establish correlations between mating system and its potential determinants, such as physiological, ecological and life history characters.

## § 1.3 Review of Previous Studies Related to Mating System of Peromyscus leucopus

The study of the mating system of Peromyscus leucopus has been hampered by their nocturnal and secretive habits, which makes it difficult to observe their behavioural interaction directly under natural conditions. As a consequence, the mating system of natural populations of P. leucopus has only been studied indirectly. Three approaches have been employed to investigate the mating system of natural populations of P. leucopus, i.e., observing parental care in the laboratory, monitoring the spatial association of adult males and females in the field, and genetic analysis on polymorphic loci to detect the presence of multiple paternity in single litters.

Male P. leucopus in cages exhibited paternal behaviour, such as licking, retrieving and huddling with their offspring

(Horner 1947; McCarty and Southwick 1977; Hartung and Dewsbury 1979). As natural selection will not favour paternal behaviour with uncertain paternity, these behavioural patterns appear to suggest high certainty of paternity, which implies, in case of P. leucopus, a monogamous mating system. A field study (Nicholson 1941), however, suggested that paternal care cannot happen in natural populations because adult males did not associate with young mice unless the latter were near weaning age. Of 64 nest boxes with young and at least one adult, a single adult male was found with young in five cases, and an adult male and an adult female were found with young in 11 cases. In all 16 cases, the young were at least 18 days old. Thus, results from paternal care studies are equivocal. A major defect in all previous studies on paternal behaviour, as recognized by Hartung and Dewsbury (1979), is the confinement of parents and young in the same cage and this causes difficulty in interpretation of their results. For example, it is hard to tell whether males huddling with young is due to true paternal behaviour or due to forced body contact because there is only one nest available.

Myton (1974) suggested that the social organization of natural populations of P. leucopus consists of basic social units or "family groups" each with an adult female and several adult males. Her conclusions were based on a field study of association patterns of adult P. leucopus monitored with a live-trap grid. This dispersion pattern would seem to suggest either simultaneous polyandry or serial polyandry. Subsequent studies on spatial association between adults of opposite sexes, however, seem to favour monogamy. Two radio-telemetry studies indicated either pair activity in adults of opposite sexes (Mineau and Madison 1977) or male-female association (Wolff and Hurlbutt 1982).

The first genetic analysis relating to mating systems in P. leucopus was reported by Wolff in an informal communication in 1986 (Peromyscus Newsletter, No.1). Wolff reported genetic evidence that adults of both sexes in natural populations of P. leucopus mated promiscuously with up to 33% of litters showing multiple paternity. If females indeed mate promiscuously, then certainty of paternity for males would be low and males should not provide paternal care, which contradicts results of Horner (1947), McCarty and Southwick (1977) and Hartung and Dewsbury (1979). Wolff, however, did not publish his genetic evidence of multiple paternity in single litters, and he recently (Cicirello and Wolff, in press) concluded that the mating system of P. leucopus was polygynous on the basis that adult males have home ranges overlapping that of several adult females.

In summary, results from previous studies on mating systems in natural populations of P. leucopus are inconsistent or even contradictory.

### § 1.4 Theoretical Considerations

To formulate a hypothesis that makes sense of those seemingly inconsistent and contradictory results from previous studies, I was forced to consider the possibility of seasonal variation in mating system. My scenario was as follows. At the onset of the breeding season, intense movements occur in overwintered animals of both sexes (Stickel 1968), resulting in a high probability that adult males and females will meet. This will cause synchronous breeding activities because oestrus synchrony in females can be induced by a sexually active male or an oestrous female in many mammalian species (Bronson and Marsden 1964; Vandenbergh 1983; Bronson 1983), including Peromyscus. Oestrus synchrony results in an overabundance of mates for some males and these males, with an overabundance of mates and having no young for them to provide paternal care to, should mate polygynously and may experience sperm-depletion. In male rats mated to satiety, semen production was not fully recovered even after 6 days of rest (Beach and Jordan 1956; Pessah and Kochva 1975; Jackson and Dewsbury 1979). Dewsbury (1984a; 1984b) found that male deer mice lost much of their inseminating power after a few consecutive copulations, whereby their vigour in copulation could induce pseudopregnancy in females. The duration of pseudopregnancy in Cricetidae and Muridae rodents is about 2 weeks (Conaway 1971), which represents a significant portion of a limited breeding season. One strategy against pseudopregnancy would be to mate with more than one male so as to increase the likelihood of mating with at least one "fresh" male. Alternatively, a female may protect herself against pseudopregnancy by forming a strong pair bond with a chosen male. This strategy would not work at the beginning of the breeding season for the following reason. If males differ genetically in their mating potential, then it is evolutionarily disadvantageous for those males with great mating potential to stay with a single female when there are many females coming into oestrus. Consequently, those males that are "fresh" would be those that have low mating potential (low quality) and natural selection would not favour females that mate with males of low quality. Thus, animals of both sexes should mate promiscuously during the early breeding season.

The formation of pair-bonds between males and females should depend on whether pair bonding will increase reproductive success of both parties involved. In P. leucopus. there are a variety of ways in which a male can provide effective paternal care. For example, due to the high energy demand of lactation, lactating females have to leave the nest frequently to feed (Harland 1979), but the development of homeothermy in juvenile Peromyscus leucopus takes about 2 weeks to complete (Hill 1972). The body temperature of the young decreases as soon as they are left alone. Thus the benefit of having a male huddling the young while the female is out feeding seems obvious. As another example, Wolff (1985a, 1986b) has reported conspecific infanticide in P.

leucopus and proposed maternal aggression as a deterrent to infanticide. As the mother has to leave the nest to forage, aggression by her would not be effective unless a defensive male is in the nest while she is out, i.e., the presence of a protective male would increase the survival of young by reducing risk of infanticide. Other kinds of help a male can potentially provide include nest building and maintenance (Nicholson 1941; McCarty and Southwick 1977), keeping young clean by grooming and licking (Horner 1947; McCarty and Southwick 1977; Hartung and Dewsbury 1979), and retrieving young when they venture out of the nest (Horner 1947; McCarty and Southwick 1977; Hartung and Dewsbury 1979). Thus, pair bonding can potentially benefit the female. Indeed females have been found to exhibit behavioral patterns that may lead to a reduction of potential mates, resulting in increased certainty of paternity. For example, females have been observed to be highly aggressive towards strange males during late pregnancy and early lactation in many rodent species including P. leucopus (King 1963; Noirot 1972; Svare 1981; Ostermeyer 1983; Wolff 1985a). These behavioural patterns in female P. leucopus would increase evolutionary advantage of pair-bonding and paternal care.

From a male's point of view, his reproductive success relative to that of other males is unlikely to increase through his paternal care to the first litter because of its uncertain paternity. There are, however, two potential benefits for a male to stay with a female. First, if females choose only familiar males as mates, then a male unfamiliar to any female will have little access to mating resources and his reproductive success should be lower than those that have established familiarity with females. This may be particularly true for Peromyscine species because a strange male is not effective in producing pregnancy blockage (Dewsbury 1985). Because a male spending all his time with a female will result in greater familiarity with her than a male that will devote only a fraction of his time to her, the female may choose only the most familiar male for postpartum copulations. This may eventually lead to the formation of pair bond.

Given a male staying with a female, there are some factors favouring paternal care. First, daughters of the first litter, although of uncertain paternity, can serve as mates for the "father". Unlike juvenile males that disperse soon after weaning, juvenile females usually breed within their mother's home range. Both Nicholson (1941) and Wolff and Lundy (1985) reported occasional pairing of the presumed father and one or two of his daughters. In a sense, paternal care for the first litter may be interpreted as the male's way of "guarding" his mating resources. Secondly, because of the high energy demands on mothers during lactation (Millar 1978; 1979), females that are both pregnant and lactating require a longer time (1 to 12 days longer) to accomplish gestation (Svihla 1932). Sometimes the female may simply give up the second litter as indicated by the regressed embryos in the uterus. With paternal care, the female may be less energetically stressed and the second litter can thus be born earlier and healthier. All these would increase the reproductive success of the male and paternal care should therefore be selected for.

In summary, both sexes of *P. leucopus* should mate promiscuously at the beginning of the breeding season, but practise monogamy thereafter. Under such a system, paternal care is favoured by natural selection. This hypothesis unifies contradictory results of previous studies and provide a consistent explanation to what have been reported of mating systems of *P. leucopus*.

On the basis of this hypothesis, four predictions can be made and tested. First, females should reject strange males during her postpartum oestrus and accept familiar males as mates. Second, males should provide paternal care. Third, there should be pair activities soon after the beginning of the breeding season. Fourth, multiple paternity in single litters should be found only in litters conceived at the beginning of the breeding season. Some of these predictions were subsequently modified with the progress of my study.

# CHAPTER 2 POSTPARTUM COPULATION

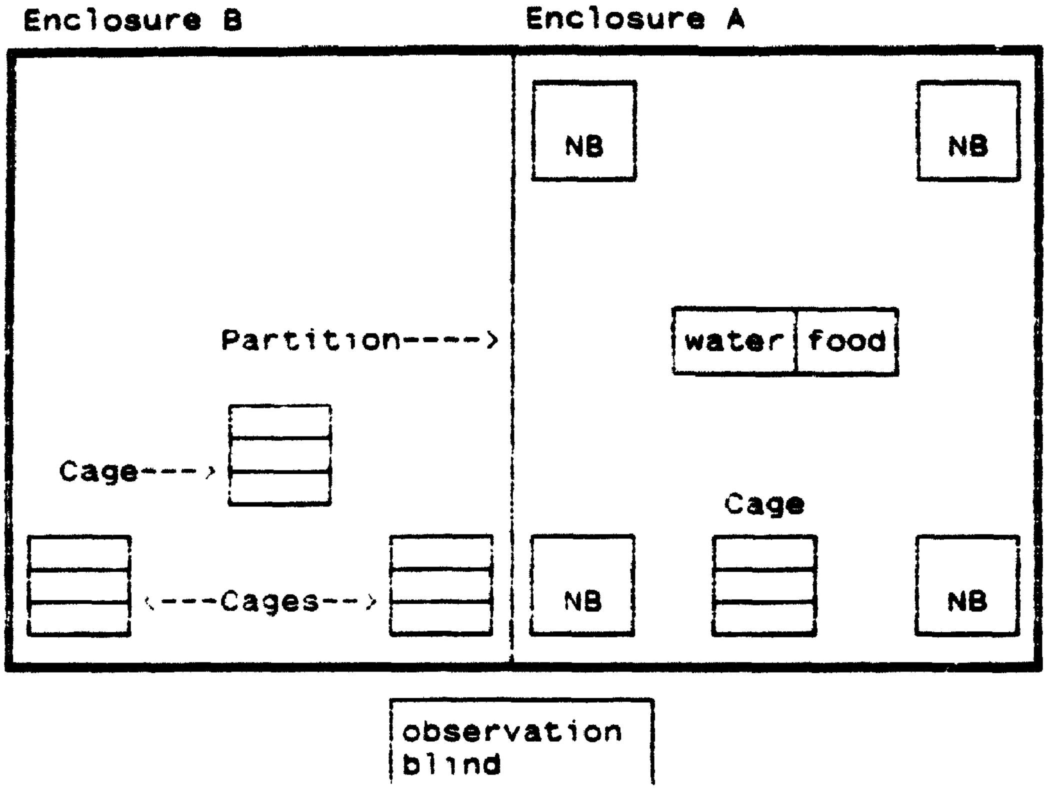
This chapter focuses on my first prediction, i.e., whether females in her postpartum oestrus reject strange males and accept familiar males as mates. All experimental females and most of experimental males for this study were from a forest in Elginfield, 28 km north-east of London, Ontario, Canada (43°N, 81°S).

## § 2.1 Method

Two in-room enclosures (2.4 m long, 1.8 m wide, and 0.8 m high, open at the top), were constructed of wood and iron partitions and arranged side by side, as shown in Figure 2-1. Enclosure A contained four numbered nest boxes (21.5 x 14.5 x 13.5 cm, inside dimensions) occupying the four corners. Water and Purina rat chow were supplied ad libitum at the centre of the enclosure in two plastic trays. Cotton batting were scattered around the water and food supplies. A light regime of 17L:7D was used, with lights off at 6:00 p.m. Dawn and dusk were simulated by manually decreasing or increasing the resistance of the light bulb circuit for about 0.5 h. A dim red light was always left on for nocturnal observations behind the blind (Fig. 2-1).

Wild-caught mice were maintained in 28 x 16 x 12 cm plastic cages, and provided with hardwood sawdust and cotton batting, and ad libitum Purina rat chow and water. Females in different stages of pregnancy, estimated by back-dating from parturition date, were paired with field caught adult males. Most pairings were not successful as females were aggressive

Fig. 2-1. Configuration of enclosures for the experiment testing whether females in postpartum oestrous reject strange males and accept familiar males as mates. The paired mice were transferred with their cage into enclosure A, and 3 strange males were transferred with their individual cages into enclosure B. The cages were covered incompletely with a wooden board so that animals can get out of the cage. NB stands for nest box.


Enclosure B
Enclosure A
NB
NB
Partition----->
water
food
Cage--->
Cage
<---Cages-->
NB
NB
observation
blind

towards males. The tail of two males was bitten off by the female they were paired with. Males that were successfully paired with females were considered as "familiar" males and were not ear-tagged. They were housed in a room separate from the room housing the "strange" males.

When the paired female was in late pregnancy (2 to 5 days to parturition, known by backdating from birth), the pair was transferred into enclosure A in the cage in which they had been housed. with the cover of the cage partially opened to allow access to the enclosure. Parturition was checked every afternoon. Three strange males, one with left ear tagged, one with right ear tagged and one with both ears tagged, were introduced into enclosure B together with their individual cages one day after the pair were introduced into enclosure A, but the cover of their cages was not lifted until the day of parturition. Birth always occurred during the day. The partition separating enclosures A and B was lifted at 4:00 p.m. to allow mice to move freely between the two enclosures. I recorded whether "strange" males were involved in postpartum copulation from behind the blind.

## § 2.2 Results

There were 6 successful pairings (out of 14) where the female was less aggressive and allowed the male to stay in the same nest. Body weight of males was not related to female aggressiveness, and the 6 less aggressive females were not lighter than the 8 more aggressive ones in terms of postpartum weight. Days to parturition for these 6 females varied from

9 to 15 days, i.e., the minimum number of days a male had spent with his female at her parturition is 9 days.

Postpartum copulation always started at dusk around 5:50 p.m., and continued for about 0.5-1 hour. The female was almost always on the run during this period. A male would mount her on the run and the female sometimes stopped running for a few seconds. Then she would resume running and leave the male licking his genital area. At this time another male would mount the female and repeat the previous event. It is difficult to distinguish ejaculation from mounting behaviourally. Dewsbury (1979) observed that ejaculation in P. maniculatus are often followed by a set of short, choppy backward steps on the dismount, but I failed to see this behavioural pattern in P. leucopus. Thus a male licking his genital area after mounting was arbitrarily considered to have ejaculated. Mounting did not always lead to ejaculation.

The recorded number of mountings and ejaculations by different males (Table 2-1) was less than what actually took place. This is due to the difficulty in instantly identifying each mounting male because all mice were darting back and forth very quickly. When I was trying to identify a male that had just mounted, several mountings and ejaculations could have taken place by other males. Also, I failed to find an objective method in recording and I do not know if I paid more attention to a particular male or not. As a consequence, it was inappropriate to use the numbers recorded in Table 2-1 to evaluate the relative benefits of being familiar to the

Table 2-1. Number of recorded mountings and ejaculations by the "familiar" male and three "strange" males.

| No. of recorded |  | No. of Tests |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
| mountings |  | 1 | 2 | 3 | 4 | 5 | 6 |
| Familiar |  | 6 | 5 | 7 | 4 | 5 | 6 |
|  | 1 | 3 | 8 | 5 | 3 | 0 | 2 |
| Strange | 2 | 7 | 4 | 6 | 4 | 5 | 4 |
|  | 3 | 4 | 2 | 4 | 6 | 7 | 7 |

| No. of recorded |  | No. of Pairs |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
| ejaculations |  | 1 | 2 | 3 | 4 | 5 | 6 |
| Familiar |  | 4 | 2 | 2 | 2 | 3 | 3 |
|  | 1 | 2 | 2 | 3 | 1 | 0 | 0 |
| Strange | 2 | 2 | 1 | 3 | 2 | 1 | 2 |
|  | 3 | 1 | 1 | 2 | 2 | 3 | 2 |

female. What is demonstrated, and I considered to be important, is the tendency of the female to accept multiple inseminations from more than one male, regardless of the fact that she had been paired with a male for at least 9 days.

Interference among males did occur during the copulation episode. When a male was mounting the female on the run, another male sometimes ran toward the female from one side. The female would suddenly change her direction of running and the mounting male would be thrown off her back. Such a thrown-off male would not lick his genital area, indicating a failure in ejaculation. No male, neither the "familiar" nor the three "strange" males displayed any overt aggressive behaviour or body contact with other males.

One "strange" male was not involved in postpartum copulation (5th pair, Table 2-1), but this was not due to the rejection of the female, but because the male, for some unknown reasons, had remained in one nest box during the whole process of postpartum copulation.

## § 2.3 Discussion

That females, which were caught in mid- or late breeding season, mated promiscuously during her postpartum oestrus was not expected from my hypothesis, which predicted that females should mate monogamously except at the beginning of the breeding season. Although I was not sure at this point whether this multiple-male mating would happen in the natural habitats of *P. leucopus*, the results prompted me to reconsider the reasoning which led to my hypothesis. Two weak links were identified. First, I was misled by studies claiming that females were very aggressive toward strange males during late pregnancy and early lactation (King 1963; Noirot 1972; Svare 1981; Ostermeyer 1983; Wolff 1985a) and such wording as "postpartum aggression" (Wolff 1985a, 1989). I thought that this aggression was continuous from late pregnancy to early lactation and that this aggression would discourage strange males, reduce the number of potential mates of each female and increase paternity. Unfortunately, this aggression is not continuous from late pregnancy to early lactation. The aggression of the female disappeared completely during her postpartum oestrus, when she appeared to accept mounting and ejaculation from any male that was capable of doing so. In other words, the high degree of aggressiveness of a female during her late pregnancy and early lactation has little to do with reduction of the number of her potential mates and increase of certainty of paternity.

The second weak link may be due to my negligence of high mortality in _P. leucopus_ (Lackey _et al._ 1985). The estimated weekly disappearance rate for adult males in natural populations of _P. leucopus_ is 0.31 (Harland _et al._ 1979). If disappearance is mainly due to mortality, then most males would not survive long enough to maintain stable pair-bonding relationship with females. In other words, females should not depend on males providing paternal care because of the low survival in adult males.

The only argument I can think of in defence of my original hypothesis is that my sample is biased because I did not have information on mating behaviour of those highly aggressive females. This argument was discarded after I tested 4 highly aggressive females under similar conditions, except that a familiar male was not involved. All females accepted insemination from at least 2 of the 3 strange males. Thus, it is generally true that females mate polyandrously, at least under my experimental conditions.

I do not, at this point, have any satisfactory explanation about why females should accept multiple inseminations from more than one male. It may be because the female cannot judge male quality, or because she cannot tell which males are sperm-depleted. A third explanation is that, if the female's reproductive system could select good sperm against bad ones, a large sperm pool contributed by many males would be better to select from than a small sperm pool contributed by a single male. To date, there has been no one testing the presence of a selection mechanism on sperm in _P. leucopus_. Wolff (1989) provided still another alternative explanation. He found that males copulated with a female were less infanticidal to her young than males unmated to her (Wolff and Cicirello, in press), and suggested that multiple insemination served the function of reducing infanticidal risk for the young (Wolff 1989). I tried to confirm this hypothesis by introducing 13 adult males each into one of 13 different litters less than 6 days old and with the mother removed. None of the males had previously copulated with the mother. I examined the 13 litters for evidence of infanticide 5 hours later and found none. Thus Wolff's infanticide hypothesis does not appear applicable to this particular population under laboratory condition.

It is puzzling that copulations took place almost entirely outside of nest boxes because there appears to be two obvious disadvantages. First, with all the participants running back and forth, the mating scene is conspicuous to a human observer and, presumably, conspicuous to a predator as well. Cushing's (1985) study on predation by least weasel, Mustela nivalis, on P. maniculatus provided a potential answer to the conspicuous copulation behaviour in P. leucopus. Cushing found the mean time of emergence of the weasel from nests in Michigan to be 152 minutes after sunset. In my study, "dusk" started at 5:30 pm and light, except for the red light for nocturnal observation, went off at 6:00 pm. Mating started around 5:50pm and lasted for about 0.5-1 hour. If the light-off corresponds to sunset, then copulation would have ended long before weasels come out of their nests.

The other possible disadvantage for the female is that, with participating males witnessing other males copulating, the female is less likely to obtain paternal care. This is difficult to explain unless paternal care is unimportant. Such "honest" promiscuity in females has never been observed in any animal species with paternal care.

In summary, females did not reject strange males and accept familiar males as mates. Thus, if certainty of paternity is low at the beginning of the breeding season, it will continue to be so. Assuming that this is what happens in nature, there is then little ground for expecting paternal care, and the well-developed paternal behaviour documented by previous studies (Horner 1947; McCarty and Southwick 1977; Hartung and Dewsbury 1979) is likely unnatural behaviour due to confined conditions.

# CHAPTER 3 PATERNAL CARE

On the basis of my results on behaviour of females during their postpartum oestrus, monogamy does not seem to be tenable and those paternal behavioural patterns documented by previous workers (Horner 1947; McCarty and Southwick 1977; Hartung and Dewsbury 1979) become difficult to explain. As Hartung and Dewsbury (1979) recognized, a major defect of all studies on paternal care in P. leucopus is the confinement of parents and young in the same cage. Paternal behaviour observed under such confined conditions may represent behaviour that does not occur naturally. This chapter examines this possibility via an experiment in which I provided a large enclosure where sires did not have to live in the same cage with dams.

## § 3.1 Methods

Enclosure A described in section 1 of chapter 2 was used for this experiment. The same light regime as in Chapter 2 was used and the same red light was used for nocturnal observations. The mice could leave the enclosure by climbing up one corner of the enclosure, which gave them access to a 3.2 x 4.5 m room. Neither food nor water was provided outside the enclosure, and mice could easily reenter the enclosure by climbing one of several supporting struts. It was easier to get into the enclosure than to get out.

Wild-caught mice were maintained in 28 x 16 x 12 cm cages, and provided with hardwood sawdust and cotton batting, and *ad libitum* Purina rat chow and water. These mice (15 males and 13 females) were used in the experiment after they had been held in captivity for more than 50 days; their field-conceived offspring were used when they were older than 90 days. Adult males and females were paired at random. The extra two adult males were paired with two randomly selected offspring females. These 15 pairs were maintained in standard cages until females were in the late stages of pregnancy or had given birth. At that time, pairs were transferred to the enclosure in the cages in which they had been housed, with the cover partially removed to allow access to the enclosure. One field-caught female was barren and thus only 14 pairs were used for the experiment. Seven pairs of mice (group 1) were transferred when their young were 4 to 7 days old, and another 7 pairs (group 2) were transferred when the female was still pregnant. Each of the 14 pairs was tested separately. Males were ear-tagged to distinguish male and female under the dim red light. All males were true fathers of the young conceived by the females.

Whole-night (7-h) observations were made from behind a blind after the mice were transferred to the enclosure, with two breaks of 20 min each night. Observation over five consecutive nights was made for group 1, and over five to seven consecutive nights for group 2 (all prepartum nights and three consecutive postpartum nights). The movement patterns of the male and the female among nest boxes were recorded. Data that were recorded include (i) nest location of the male, female, and nestlings during the test; (ii) frequency with which different nest boxes were entered by the male and the female, excluding entrances made to cache food, build new nests, or avoid aggression by the other adult; (iii) frequency with which nest material and young were carried by the male and by the female; (iv) frequency of caching food by the male and by the female; and (v) time spent (nearest 10 s) by the male in the female's nest. To avoid confusion, a nest in this experiment is defined as a nest box containing cotton batting deposited by the tested animal and structured into a cavity and one or more entrances (or exits), which serves as day shelter or home for young. Nest sharing by the adult pair means the use of one nest by the two adults, not necessarily at the same time.

## § 3.2 Results

### General description

Adults admitted to the enclosure started exploring the enclosure around 6:15 p.m. Exploration involved checking the four edges of the enclosure, the four nest boxes, and food and water containers over a period of 50-190 min. Nest building followed exploration and involved carrying an average of 49 (N=14 females, SE=4.7) mouthfuls of nesting material into a nest box. The whole process of nest building required an average of 53 (N=14 females, SE=4.7) min of concentrated effort, after which the mice carried nesting material into a nest only sporadically.

In group 1, males came out of the cage 5-30 min earlier than did females ($p < 0.01$). Young were transferred by the female into a nest box during either the first (N=6) or second

(N=1) night, after nesting material was collected. Six females shifted their nests during the second night and one female did this during the third night. During nest shifting, the cotton from the natal nest was transferred into the new nest, with new nesting material being added later. This new nest was used during the remaining observation periods and no other nest was build. Males in group 1 built their nests after the females built their first nest. The quantity of cotton batting in a male's nest was less than a quarter of that of a female's nest. Males did not shift their nests unless the female happened to shift her nest to his. This occurred twice. In both cases the male built a new nest in another nest box.

In group 2, males and females started exploration at similar times and there was no detectable difference in which sex came out of the cage first. Two of the seven group 2 males initiated nest building 20-25 min after introduction to the enclosure. In all seven pairs, males and females shared the first nest. However, all seven females in group 2 gave birth in a different nest box and transferred the young either back to the old nest box (N=5) or to a third nest (N=2). All births occurred during the day and postpartum oestrus occurred the same evening about 10-25 min after "sunset". The mating lasted an average of 43 (N=7, SE=8.2) min during which the male continuously chased the female; an average of 23 (N=7, SE=3.8) copulations (mountings) were observed outside of nest boxes. No copulation longer than 10 s was observed. Copulation may also have occurred within the nest boxes because the male occasionally chased the female into one of the nest boxes (except for the female's nest which had never been entered during copulation) for up to 40 s, which is much longer than required for a copulation.

Two females in group 1 and three females in group 2 that had given birth were very aggressive towards their mates and actively searched for and chased them throughout most of their activity period. Another three females in group 1 were aggressive only when the males attempted to enter their nests at dawn. These females either blocked the entrance of the nest box with forefeet or rushed out of the nest box to drive the male away. They did not actively search for and chase the male. No male was aggressive towards females.

### Paternal care

Males shared nests with females when they were pregnant, but not when they were lactating ($P < 0.0001$, Table 3-1). Each male in group 2 built his own nest within 2 days of postpartum copulation. Eight males left the enclosure, built nests outside, and stopped visiting nest boxes in the enclosure. Only twice did I observe these males entering the enclosure for water in the evening; these mice may have entered the enclosure for food and water during the day, when I was absent. This "male desertion" occurred only after the postpartum oestrus of the female. The relationship between male desertion and female reproductive status ($X^2$-test with Yates' correction, $p = 0.039$) is shown in Table 3-2. No pregnant female left the enclosure, but two lactating females

Table 3-1. Nest sharing in relation to the female's reproductive status.

|  | Female's reproductive status |  |  |
|---|---|---|---|
| Nest Sharing | Pregnant | Lactating (group 1) | Lactating (group 2) |
| Yes | 7 | 0 | 0 |
| No | 0 | 7 | 7 |

did. The latter returned to the enclosure within 20 min.

Table 3-3 presents the number of entries made by males and females into the male's nest box, the female's nest box (with young), and the other two nest boxes. Because of the tendency to move nests, data in Table 3-3 were recorded only on the third and fourth nights after the introduction for group 1, and on the second and third nights after parturition for group 2. Males rarely entered the female's nest with young (Table 3-3, N=6, mean = 0.58 times; the upper limit of 95% of confidence interval was 1.35 times), which precluded paternal care such as licking, retrieving, or huddling with the young. Moreover, even if a male did enter his female's nest box (7 times altogether; Table 3-3), the duration of his stay never exceeded 2 min; the three periods long enough to permit recording were 10, 30 and 90 s.

These results show that direct paternal care did not occur, but males could still make indirect parental investments by building new nests or caching food for the female. Seventeen cases of nest shifting, involving 13 pairs (4 pairs moved nests twice) were recorded when both the male and the female were in the enclosure. In all cases the female did all of the nest building and transferred all of the young.

There were great differences in food caching behaviour between males and females when both the male and the female were in the enclosure (Table 3-4). While all females were active food hoarders, males rarely hoarded. There was no indication that the male cached food for the female.

Table 3-2. Relationship between male desertion[1] and female reproductive status.

| Male Desertion | Female's reproductive status | | |
|---|---|---|---|
| | Pregnant | Lactating (group 1)[2] | Lactating (group 2)[3] |
| Yes | 0 | 5 | 3 |
| No | 7 | 2 | 4 |

Note $X^2$-test with Yates' correction, df = 1, p = 0.039.

1. The male nested outside the enclosure.
2. Data of first two nights after introduction to enclosure.
3. Data of first two nights after parturition.

Table 3-3. Mean frequency (number of times per night) of the male and female entering different nest boxes (only pairs in which the male did not desert the enclosure are included).

| | Male's nest | | Female's nest | | Other nests | | No. of |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | nights |
| Group 1 | | | | | | | |
| Pair 1 | 14 | 8.5 | 0.5 | 8.5 | 22 | 27 | 2 |
| Pair 2 | 7 | 28 | 1.5 | 7.5 | 11 | 47 | 2 |
| Group 2 | | | | | | | |
| Pair 3 | 6.5 | 24 | 1.5 | 7.5 | 6 | 22.5 | 2 |
| 4 | 4 | 10 | 0 | 11.5 | 2 | 8 | 2 |
| 5 | 2.5 | 9 | 0 | 10 | 1 | 5.5 | 2 |
| 6 | 4 | 9 | 0 | 8 | 1 | 10 | 1[1] |
| Mean | 6.3 | 14.2 | 0.6[2] | 8.4 | 7.3 | 20 | |

[1] Male deserted the following night.

[2] Upper limit of 95% confidence interval is 1.35.

Table 3-4. Differences between sexes in food-caching activity.

| Pair No. | Female reprod. status | No. of caching trips by male | No. of caching trips by female | No. of nights |
|---|---|---|---|---|
| 1 | Lactating | 0 | 70 | 2 |
| 2 | Lactating | 0 | 89 | 3 |
| 3 | Lactating | 1 | 87 | 2 |
| 4 | Pregnant | 0 | 123 | 3 |
| 5 | Lactating | 0 | 122 | 2 |
| 6 | Lactating | 0 | 13 | 1 |
| 7 | Lactating | 2 | 84 | 1 |

## § 3.3 Discussion

Whether a male should provide paternal care depends on fitness gained by such behaviour relative to fitness gained by extra matings (Trivers 1972; Emlen and Oring 1977; Kleiman and Malcolm 1981; Elwood 1983; Dewsbury 1985). If paternal behaviour was triggered by proximate factors, then my experimental setup favoured paternal behaviour in two ways relative to natural conditions. First, the males in my experiment have 100% certainty of paternity as females were paired with males before pregnancy. Such a high certainty of paternity may be unlikely in the field because of the female's promiscuous tendency. Second, in my experiment there were no females available to the males other than their mates, i.e. probability of having extra matings was zero. This would also be unlikely under natural conditions. Because males in my experiment showed no paternal care, even with full paternity and no chance of extra matings, I infer that paternal care is very unlikely in nature. The fact that 5 of 14 females were aggressive towards their mates and another 3 actively prevented their mates from entering their nests suggests that females do not tolerate paternal involvement in raising the young. Thus the opportunity for the male to increase fitness by providing paternal care appears to be non-existent; males should seek extra matings instead of providing paternal care.

Why some females were aggressive towards their mates is not clear. Wolff (1985a, 1986b) and Wolff and Cicirello (in press) found infanticide by conspecific P. leucopus when young were 1-12 days old, and suggested that maternal aggression was protection against infanticide. However, aggression persists well past the first 2 weeks of lactation. Wolff (1985a) found that young older than 17 days were not susceptible to infanticide, yet Gleason _et al._ (1980) found a high degree of maternal aggression when young were 20 days old. If maternal aggression is for protection against infanticide, then there is no point in maternal aggression when young are no longer susceptible to infanticide. Alternatively, maternal aggression may also be explained in terms of resource defence for securing a sufficient energy supply during lactation. The male may incur a cost to the female by consuming food cached by her. This cached food may serve to buffer the unpredictability of dispersed food resources in natural environments and may be important in meeting the high energy demands of breeding females (Millar 1978; 1979).

Sex differences in food caching by _P. leucopus_ are logical. given other behavioural patterns of males and females. First, caching food is for future use, which implies that the cacher is a long-term resident. Second, cachers must be able to defend the cached food against theft (Smith and Reichman 1984), which implies that the cacher is territorial. Nel (1975) found that caching behaviour was associated with solitary, territorial habits when he compared the hoarding behaviour of nine species of Kalahari Desert rodents. Several studies indicate that adult female _P. leucopus_ are solitary and territorial, while adult males are not (Nicholson 1941;

Stickel 1968; Metzgar 1971). This observation may be related to the fact that females must secure a stable energy supply for raising young; males in a promiscuous mating system should shift their home ranges according to the location of females approaching oestrus. Thus, females should be the food-caching sex in P. leucopus.

Bowers and Smith (1979) found differential use of microhabitats between adult male and female deer mice during the breeding season, with females occupying more favourable microhabitats and having smaller home ranges than males. They suggested that, because the home range of males was of poorer quality than that of females, males need a larger area to meet their daily requirements. They did not justify the implicit assumption that males and females have similar daily requirement. That females occupied microhabitats better than that of males was interpreted as altruistic behaviour of males, as they stated that "Such a sacrifice [in males] is not conceptually different from male weasels and songbirds bringing food to their mate and dependent young". A more likely explanation for differences in home range size between the sexes would be that a male has to visit different females for mating purposes and consequently has a home range larger than that of a female. The difference in microhabitat quality between sexes is also indicated in my experiment. Eight males in my experiment nested outside of the enclosure, i.e., in a "microhabitat" that has neither food nor water. But this differences in microhabitat quality between the sexes may not be due to any kind of sacrifice in the male part, but instead results from the females' territorial aggression and the males' lack of interest in microhabitat quality in terms of food, water and shelter. Bowers and Smith's (1979) finding of sexual differences in microhabitat quality indicates the lack of paternal care because, if males stay with females in caring for young, they would be occupying the same microhabitat and there would be no sexual differences in microhabitat quality.

I assumed in my experiment that food abundance would not alter paternal behaviour, but this assumption requires justification. Scarce food can have two consequences. First, lactating females may be energetically stressed, increasing the importance of paternal care. Second, oestrous females would be spatially and temporally rare, i.e., probability of remating for males is low. Both of these consequences could favour monogamy and paternal care. I do not know if males are able to adjust their behaviour according to the need for paternal care. If they are, then the *ad libitum* food supply in my experiment may have provided males with a cue that paternal care was not needed. Two lines of evidence, however, favour my assumption. First, in paternal care studies with caged mice (McCarty and Southwick 1977; Hartung and Dewsbury 1979), food was also provided *ad libitum*, but paternal care was common, i.e., *ad libitum* food did not inhibit paternal behaviour. Second, food resources for these mice may indeed be abundant during the breeding season because supplementary food did not increase breeding activities (Hansen and Batzli

1978; Wolff 1986). In conclusion, these results suggest that paternal care is unlikely in natural populations of *P. leucopus*.

Given the previous results that females mate promiscuously and that males do not provide paternal care, Myton's (1974) study on spatial relationships between adults of opposite sex appears logical. Myton (1974) found the social organization of *P. leucopus* to consist of basic social units or "family groups" each with one adult female and several adult males. My interpretation is that the female in Myton's basic social unit is probably one close to oestrus and several adult males are around her waiting for the coming copulation. In other words, the basic social unit is temporary and males will move away to form new "basic social unit" with other females that are coming into oestrus.

# CHAPTER 4 SPATIAL ASSOCIATION

Because the previous results from my laboratory experiments suggest that adult males change their behaviour and spatial relationship to females in response to the female's reproductive status, this experiment was designed to test whether adult males in natural populations of P. leucopus adjust their spatial position in relation to females' breeding status as well, i.e., whether females close to oestrus have more adult males around her than females far from oestrus. Female P. leucopus are usually receptive soon after the birth of a litter, with birth occurring during the day and postpartum oestrus starting in dusk of the same day (Svihla 1932; pers. obs.). If females mate promiscuously and males are only interested in females approaching oestrus, then a female near parturition (postpartum oestrus) should attract more adult males than a female in early pregnancy. This pattern has been observed in enclosures (Table 3-1, 3-2 and 3-3), but whether this happens in nature requires confirmation.

## § 4.1 Method

Mouse populations in five forests near London, Ontario, were monitored using grids and lines of Longworth live traps from May 15 to August 26, 1987 (Table 4-1). All habitats sampled had similar physiognomic features. Traps were placed 10 m apart within lines, and lines were 15 m apart in grids. A trapline or grid was considered as a sampling unit if traps from other lines or grids were at least 60 m away from this line or grid. The number of sampling units in each of the five

Table 4-1. Monitoring scheme of *Peromyscus leucopus* in the five forests.

| Forests | Monitoring period | pregnant females caught | No. of sampling unit | total No. of traps |
|---|---|---|---|---|
| Arva | May 15-June 3 | 8 | 5 | 108 |
| Weldon | June 3-June 10 | 6 | 2 | 100 |
| McLarty's | June 15-July 5 | 5 | 1 | 78 |
| Burm's | July 5-July 26 | 8 | 3 | 140 |
| Hwy. 16 | July 27-Aug. 26 | 8 | 2 | 90 |

forests is also included in Table 4-1. Sampling units differed much in size among, but not within, the five forests. All traps were baited with sunflower seeds, provided with cotton batting, and checked daily for at least 5 days. Animals caught were ear tagged, and sex, age (adult or juvenile), and location were recorded. Adult females were brought back to the laboratory and checked for parturition every 3-4 days. Number of days to parturition was recorded for each female. Traps within 30 m of the trap where each female was caught were arbitrarily defined as her neighbourhood traps. Figure 4-1 illustrates the number of neighbourhood traps for two females (F1 and F2) in one sampling unit which is a grid of six lines (A-F) each with 13 traps. The female F1 has 22 neighbourhood traps while female F2 has only eight neighbourhood traps. Females caught on traplines had a maximum of six neighbourhood traps. If two females (e.g., F1 and F3 in Fig. 4-1) were caught within 60 m, some neighbourhood traps were common to both females. Such a "common" trap was counted as 0.5 traps for each female. Adult males caught in a neighbourhood trap of a female were recorded as her neighbourhood males. A male caught in a "common" trap was counted as 0.5 neighbourhood males for each female. Because the movement of males may be influenced by the removal of their neighbouring females, only males caught up to the night when their neighbouring females were caught were included in the analysis. The variables recorded were (i) days to parturition ($D_p$) of each female, estimated by back-dating from birth, (ii) the distance of a

Fig. 4-1. A grid of traps with six lines (A-D) each with 13 traps, illustrating the assignment of neighbourhood traps to different females. The neighbourhood traps for female F1 and F2 are numbered.

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | ● | ● | ● | ● | ● | ● | ● | ● | ● | 1 | 2 | 3 | F2 |
| B | ● | ● | ● | ● | 12 | 13 | 14 | ● | ● | ● | 4 | 5 | 6 |
| C | ● | ● | ● | 7 | 8 | 9 | 10 | 11 | ● | ● | ● | 7 | 8 |
| D | F3 | ● | 1 | 2 | 3 | F1 | 4 | 5 | 6 | ● | ● | ● | ● |
| E | ● | ● | ● | 15 | 16 | 17 | 18 | 19 | ● | ● | ● | ● | ● |
| F | ● | ● | ● | ● | 20 | 21 | 22 | ● | ● | ● | ● | ● | ● |

female to her closest adult female (FDF), (iii) the number of neighbourhood males for each female ($N_m$), (iv) the total number of adult males caught in the sampling unit ($N_t$), (v) the number of neighbourhood traps for each female ($N_{nt}$), and (vi) the total number of traps in the sampling unit ($N_{tt}$). The main purpose of this study is to examine whether $N_m$ is negatively correlated with $D_p$.

## § 4.2 Results

Altogether 45 adult females were caught in the field, of which 35 gave birth in the laboratory. Thus most adult females in the field were pregnant. Mean and standard deviation of the six variables for the 35 females are displayed in Table 4-2.

Three factors may have influenced the number of adult males caught in a female's neighbourhood ($N_m$). First, a female with many neighbourhood traps (a large $N_{nt}$) should have had greater $N_m$ than a female with few neighbourhood traps. For example, female F1 in Fig. 4-1 should have greater $N_m$ than female F2, everything else being equal. If the distribution of adult males is random, then the expected number of neighbourhood males ($N_e$) is simply a function of $N_t$, $N_{nt}$, and $N_{tt}$, i.e.,

[1] $N_e = (N_t \times N_{nt}) \div N_{tt}$.

For example, if five males were caught in the grid depicted in Fig. 4-1, then the $N_e$ for female F1 is 1.41 (=5 x 22/78)

Table 4-2. Some descriptive statistics for the six variables and $N_e$, the expected number of neighbourhood males.

| Variables | N | Mean | S.D. |
|---|---|---|---|
| $D_p$ | 35 | 11.29 | 5.79 |
| $N_m$ | 35 | 1.09 | 1.09 |
| $N_t$ | 35 | 3.88 | 2.49 |
| $N_{nt}$ | 35 | 9.86 | 4.56 |
| $N_{tt}$ | 14 | 36.86 | 9.81 |
| FDF | 35 | 66.97 | 55.60 |
| $N_e$ | 35 | 0.99 | 0.59 |

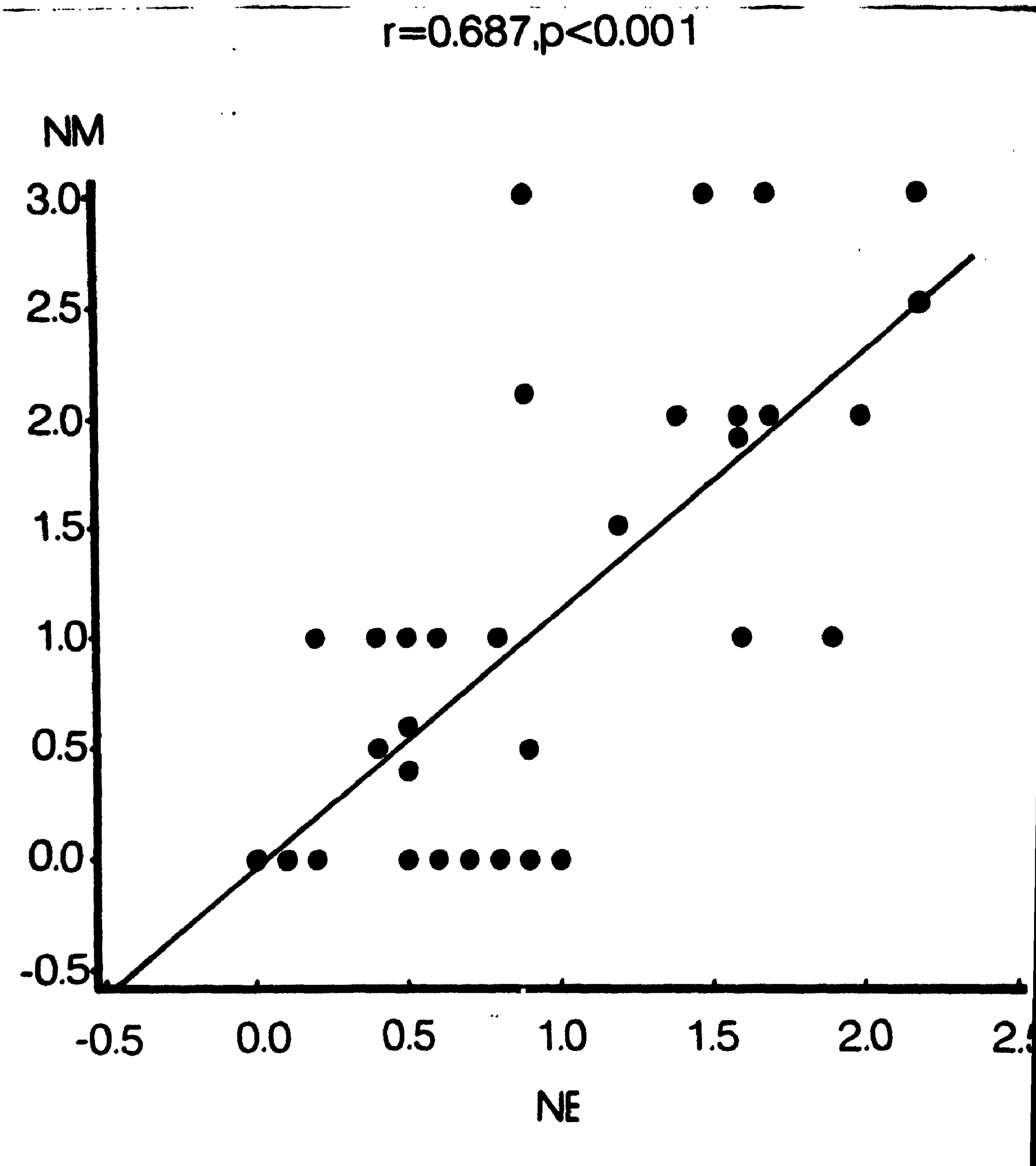
Note: see text for definitions of variable names and the calculation of $N_e$.

males. $N_e$ was calculated in this way for all 35 females. Means and standard deviation of $N_e$ are also included in Table 4-2. A truly random distribution of males in space implies that mean $N_m$ equals mean $N_e$, and $N_m$ increases with $N_e$ with slope equal to 1 and intercept equal to 0. Two factors, however, tend to draw $N_m$ away from $N_e$. One is that males and females may be spatially associated. For example, both males and females may be spatially associated with favourable microhabitats. This will result in $N_m$ increasing with $N_e$ with a slope larger than 1, i.e.,

[2] $N_m = (1 + b_{Asn})\cdot N_e$

where $b_{Asn}$ measures the degree and type of association between males and females. $b_{Asn}$ smaller than 0 indicates a negative association, and $b_{Asn}$ equal to 0 indicates that the distribution of males is independent of females. The intercept will still be 0 because $N_m = 0$ when $N_e = 0$. The observed relationship between $N_e$ and $N_m$ is shown in Fig. 4-2. The other factor that may influence $N_m$ is the male's spatial response to the female's reproductive status. For example, a female with $D_p$ of 2 may attract more males than a female with $D_p$ of 20 because males only have to wait for 2 days to mate in the

Fig. 4-2. Relationship between observed number of neighbourhood males (NM) and its expected value (NE). A simple linear regression line is shown as a solid line across the scatter plot.


r=0.687,p<0.001
NM
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-0.5
0.0
0.5
1.0
1.5
2.0
NE

former and 20 days in the latter. In short, I predicted that females close to their parturition date (small $D_p$) should have more males nearby than females in early pregnancy (large $D_p$). A regression model used to test my prediction is as follows:

[3] $N_m = b_0 + (1 + b_{Asn}) \cdot N_e + b_{Dp} \cdot D_p$

where $b_0$ is the intercept and $b_{Dp}$ measures the type of male spatial response to the female's breeding status. My prediction was that $N_m$ should increase with a decrease in $D_p$, i.e., $b_{Dp} < 0$.

Because the five areas sampled differed in density and in size of sampling units, the area effect was coded with four dummy variables and incorporated in the regression model (Berenson <u>et al.</u> 1983). The final model fitted to the data was

[4] $N_m = b_0 + 1.038 \cdot N_e - 0.056 \cdot D_p$,

where $b_0 = 0.491$ for Hwy. 16, 0.460 for Arva, 1.128 for Weldon, 0.501 for McLarty's, and 0.914 for Burm's (Table 4-3). The model accounts for 57.1% of the total variance in $N_m$, with $N_e$ accounting for 23% of the total variance and $D_p$ accounting for 7%, using sequential sum of squares (Kim and Kohout 1975; Tabachnick and Fidell 1983). The value of $b_{Asn}$ (=1.038 - 1) is close to 0, indicating little association between males and females. $D_p$ (=-0.056) is significantly smaller than 0 (Table 4-3, $t = -2.16$, $df = 28$, $p = 0.021$, one-tailed test), suggesting that adult males indeed adjust their

Table 4-3. Regression coefficients and significance tests for the regression model $N_m = b_0 + (1 + b_{Asn}) \cdot N_e + b_{Dp} \cdot D_p$.

| Variables | Coeff-icient | S.D. of Coefficient | T | P* | 95% C.L. low | 95% C.L. high |
|---|---|---|---|---|---|---|
| Intercept | 0.491 | 0.426 | 1.15 | ns | | |
| Dummy 1 | -0.031 | 0.399 | -0.08 | ns | | |
| Dummy 2 | 0.637 | 0.429 | 1.49 | ns | | |
| Dummy 3 | 0.010 | 0.460 | 0.02 | ns | | |
| Dummy 4 | 0.423 | 0.416 | 1.02 | ns | | |
| $N_e$ | 1.038 | 0.261 | 3.98 | 0.001 | 0.59 | 1.48 |
| $D_p$ | -0.056 | 0.026 | -2.16 | 0.021 | -0.10 | -0.01 |

Analysis of variance

| Source of variation | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 6 | 20.73 | 3.45 | 6.22 | < 0.01 |
| Residual | 28 | 15.56 | 0.56 | | |
| Total | 34 | 36.29 | | | |

Note: C.I.. confidence interval. Dummy variables 1 to 4 are for Arva. Weldon. McLarty's, and Burm's, respectively. Their corresponding coefficients change the intercept of the model. The intercept for Hwy. 16 is 0.491. Intercepts for the other areas are the sum of 0.491 and the respective coefficients.

$*H_0$: $b_{dummy\ var.} = 0$, $(1 + b_{Asn}) = 0$, $b_{Dp} \geq 0$.

position in relation to the stage of pregnancy of adult females.

Although the above results support my hypothesis, one experimental constraint remains unconsidered. The neighbourhood area of a female in this study is defined as the area with a radius of 30 m from the trap where the female was caught. If two females were caught close to each other, then each male caught in "common" traps was counted as 0.5 for each female; but if only one of the two females was caught, then all males caught in those potentially "common" traps were counted as her neighbourhood males. This would yield an overestimate of $N_m$ for the single female relative to the situation when both females were caught. The degree of this overestimation will decrease with the thoroughness of trapping, which is, unfortunately, difficult to estimate. Assuming that density is equal within a forest, then the trapping is likely more thorough in situations in which several females were caught close to each other than in situations where only single females were caught. The distance from a female to her closest female neighbour (FDF) is smaller in the former situation than in the latter. Therefore the smaller the FDF, the more thorough the trapping might be and the less likely that $N_m$ was overestimated. This relationship should hold until FDF is as large as 60 m, but no larger. To illustrate the last point, consider female F1 in Fig. 4-1. F1 has 22 neighbourhood traps. If another female was caught in location E6, then 14 of the 22 traps are "common" traps and only half of the males caught in these "common" traps can be counted as F1's neighbourhood males. But if no other female is caught within 120 m, the number of neighbourhood males that F1 has will still be the same as when there is no other female caught within 60 m., i.e., the number of adult males caught in those 22 traps. Thus the effect of FDF on $N_m$ disappears when FDF is larger than 60 m. For this reason, all FDF values larger than 60 m were recoded to 60. Fig. 4-3 shows the relationship between $N_m$ and FDF under these conditions. FDF does seem to influence $N_m$ (Fig. 4-3) and it is appropriate to incorporate FDF into the regression model:

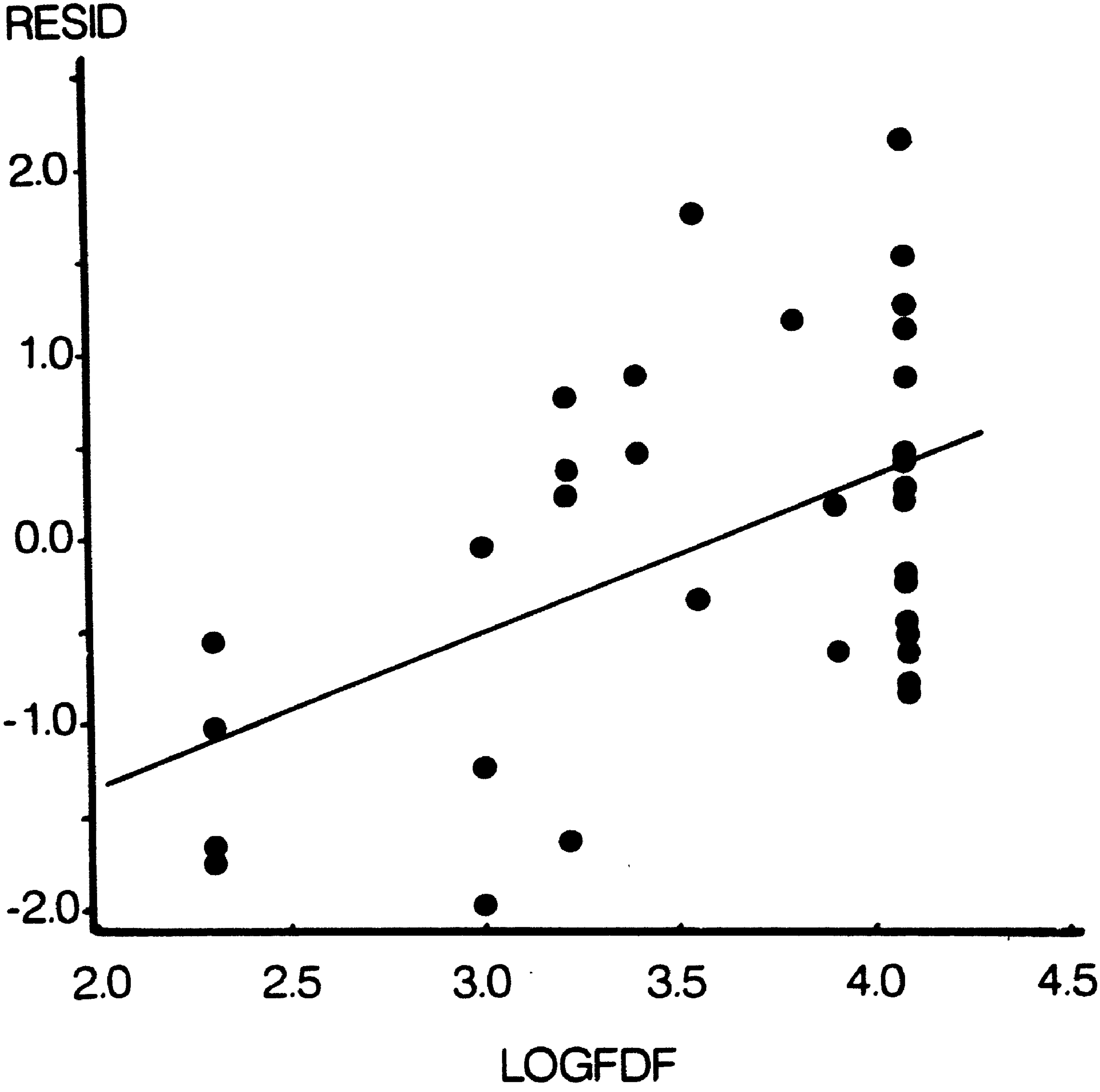
$$[5] \quad N_m = b_0 + b_{FDF} \cdot \ln FDF + (1 + b_{Asn}) \cdot N_e + b_{Dp} \cdot D_p$$

The model fitted to the data is

$$[6] \quad N_m = b_0 + 0.719 \cdot (\ln FDF) + 0.988 \cdot N_e - 0.062 \cdot D_p$$

Detailed results are presented in Table 4-4. The error variance is much reduced relative to the previous model and this model accounts for 71.6% of the total variation in $N_m$, with FDF accounting for 15.2%, $N_e$ accounting for 20.5% and $D_p$ accounting for 3.3%. $b_{Dp}$ is significantly smaller than 0 (Table 4-4, t = -2.89, df = 27, p = 0.005, one-tailed test), suggesting a strong negative relationship between $D_p$ and $N_m$. The $b_{Asn}$ (=1-0.988) is still very close to zero, implying no association between adult males and females given that other variables are controlled. Fig. 4-4 shows the relationship

Fig. 4-3. The influence of FDF on $N_m$: a plot of the residual of the model (RESID) $N_m = b_0 + (1 + b_{Asn})\cdot N_e + b_{Dp}\cdot D_p$ versus LOGFDF (natural logarithm of FDF). A simple linear regression line is shown as a solid line across the scatter plot.



r=0.504,p<0.01,one-tail test

Table 4-4. Regression coefficients and significance tests for the regression model $N_m = b_0 + b_{FDF} \cdot \ln(FDF) + (1 + b_{Asn}) \cdot N_e + b_{Dp} \cdot D_p$.

| Variables | Coefficient | S.D. of Coefficient | T | P* | 95% C.L. low | 95% C.L. high |
|---|---|---|---|---|---|---|
| Intercept | -1.885 | 0.714 | -2.64 | 0.016 | | |
| Dummy 1 | -1.551 | 0.359 | -1.53 | ns | | |
| Dummy 2 | 0.551 | 0.356 | 1.55 | ns | | |
| Dummy 3 | -0.258 | 0.388 | -0.67 | ns | | |
| Dummy 4 | 0.473 | 0.345 | 1.37 | ns | | |
| FDF | 0.719 | 0.194 | 3.71 | 0.001 | 0.39 | 1.32 |
| $N_e$ | 0.988 | 0.217 | 4.56 | 0.001 | 0.62 | 1.36 |
| $D_p$ | -0.062 | 0.021 | -2.89 | 0.005 | -0.10 | -0.03 |

Analysis of variance

| Source of variation | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 6 | 25.984 | 3.712 | 9.733 | < 0.001 |
| Residual | 28 | 10.299 | 0.381 | | |
| Total | 34 | 36.283 | | | |

Note: C.I., confidence interval. Dummy variables 1 to 4 are for Arva, Weldon, McLarty's, and Burm's, respectively. Their corresponding coefficients change the intercept of the model. The intercept for Hwy. 16 is 0.491. Intercepts for the other areas are the sum of 0.491 and the respective coefficients.

$^*H_0$: $b_{FDF} \le 0$, $b_{dummy\ var} = 0$, $(1 + b_{Asn}) = 0$, $b_{Dp} \ge 0$.

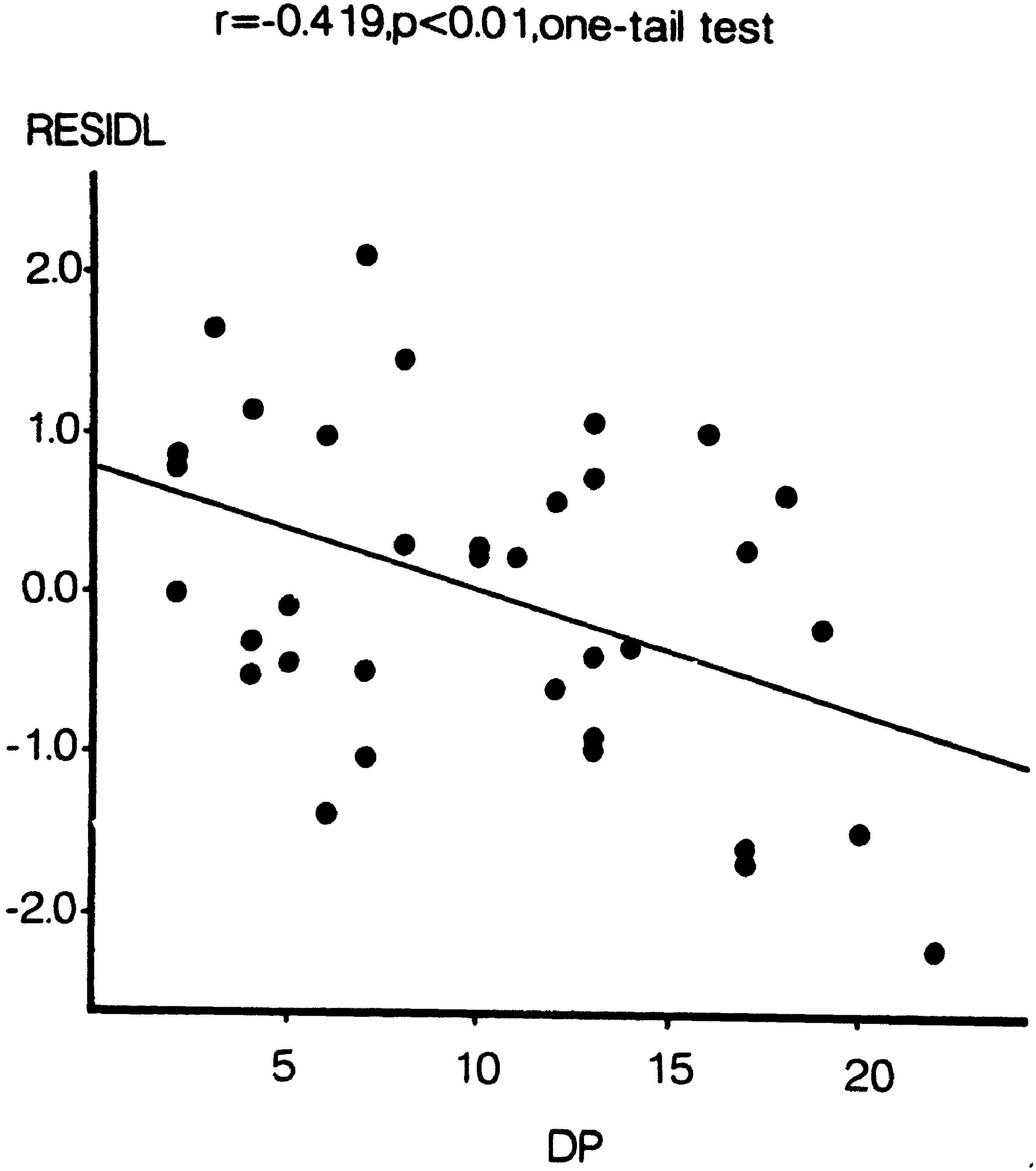
between $D_p$ and $N_m$ as follows. $D_p$ was removed from the last model and the residual of $N_m$ from that model without $D_p$ represents variation in $N_m$ that cannot be explained by differences in area, FDF, and $N_e$. This residual was plotted as a function of $D_p$ in Fig. 4-4 to demonstrate if more "extra $N_m$" corresponds to smaller $D_p$. The graphic presentation supported in a much simpler way my conclusion that the closer a female is to her postpartum oestrus, the more adult males are found in her neighbourhood.

## § 4.3 Discussion

The percentage of the total variance accounted for by $D_p$ was small (7.2% and 8.8% for the first and second regression models, respectively), which was expected because of the many sampling assumptions involved. For example, the trap where the female was caught may not have been the centre of her home range, which would result in the misassignment of neighbourhood traps and, consequently, the misassignment of neighbourhood males. In addition, when two females of very different reproductive status were caught close to each other, there would be many "common" traps and each male caught in these "common" traps was counted 0.5 for each female. In that case, the effect of $D_p$ on $N_m$, no matter how great, is much obscured. Fortunately, females were rarely caught close to each other.

The $b_{Dp}$ showing the numerical relationship between $N_m$ and

Fig. 3-4. The influence of $D_p$ on $N_m$: a plot of the residual of the model (RESID) $N_m = b_0 + b_{FDF} \cdot \ln FDF + (1 + b_{Asn}) \cdot N_e$ versus $D_p$ (DP). A simple linear regression line is shown as a solid line across the scatter plot.


r=-0.419,p<0.01,one-tail test
RESIDL
2.0
1.0
0.0
-1.0
-2.0
5
10
15
20
DP

$D_p$ was also small (-0.056 for first model and -0.062 for the second), but my model may have underestimated $b_{Dp}$. $D_p$ may not have been estimated accurately because females were not checked daily. This error associated with $D_p$ (independent variable in the regression model) would result in an underestimate of the slope (Sokal and Rohlf 1981; Snedecor and Cochran 1980). What is important is the establishment of a negative relationship between $N_m$ and $D_p$.

My study shows that a female approaching her postpartum oestrus may have several males in her neighbourhood. This may provide an explanation for the pair activity reported in Mineau and Madison (1977). If one female in late pregnancy and one of several males in her neighbourhood are radiotelemetered, this situation would appear to the observer as pair activity simply because all the other non-radiotelemetered males are undetectable. On the basis of my results, I conclude that pair activity is not the true pattern in P. leucopus and the spacing pattern of males in relation to a female's breeding status, as revealed in this study, support a promiscuous mating system suggested in previous chapters.

Myton(1974) suggested that natural populations of P. leucopus are arranged in "family" groups consisting of one adult female and several adult males. She did not state whether this "family" grouping was permanent or temporary, but this social grouping certainly would require a very male-biased sex ratio, with at least two adult males to every adult female. Such a biased sex ratio has never been established in natural populations of P. leucopus as no field studies on sex ratio of natural populations of the species controlled for differential trappability between the sexes. The sex ratio at birth is 167:155 (male:female) according to my own data, and the 95% confidence interval for the percentage of males is 0.46-0.58. The survival of juveniles in the summer, as estimated by Harland et al. (1979), is 0.69 for males and 0.72 for females, i.e., juvenile females do not suffer higher mortality than juvenile males. Thus I have no reason to believe that the operational sex ratio is highly male-biased in natural populations. For this reason, Myton's (1974) observation that some adult females have several adult males nearby necessarily implies that there must be some adult females that have just one male nearby or even no male at all. My results explain why some adult females have more males nearby than others in terms of $D_p$, and Myton's "family" groups may just be temporary associations of several adult males with an adult female close to oestrus.

That males adjust their positions in relation to the female's reproductive status may also contribute to the differential dispersal among juveniles whereby juvenile males, upon weaning, disperse while juvenile females remain in the maternal home range. This differential dispersal is traditionally explained in terms of avoiding close inbreeding

(Krohne et al. 1984; Wolff and Lundy 1985; Goundie and Vessey 1986; Wolff et al. 1988). However, the dispersal time of juvenile males is the time when they are weaned, which is much earlier than the time when they are approaching sexual maturity. Weaning corresponds to the time when the next litter is about to be born, i.e.. the time when several adult males are attracted to the dam for postpartum copulation. Juvenile males may simply be driven out of their maternal home range by these adult males. Wolff et al. (1988) argued that aggression from adult males was not responsible for dispersal of juvenile males because only a small percentage (26 and 28%) in two series of trials) of adult males showed aggression toward juveniles. Wolff et al. (1988), however, did not observe behaviour of adult male P. leucopus towards juvenile males with an oestrous female nearby. Thus adult aggression cannot yet be completely ruled out as an explanation for the differential dispersal. Adult aggression has been indicated in several studies involving a congeneric species, P. maniculatus (Sadleir 1965; Healey 1967; van Horne 1981).

I should finally mention that my results could have an alternative interpretation. The negative relationship between $D_p$ and $N_m$ could also be explained by varying degrees of female aggression during pregnancy. Females in early pregnancy (large $D_p$) may have a recently born litter, i.e., early lactation. It is known that females during the early lactation period are very aggressive toward males (Layne 1968; Gleason et al. 1980;

Wolff 1985), and I observed in enclosures that a female with newborn young actually killed an adult male that blundered into the nest containing those young. If female aggression decreases with the progress of lactation (or the decrease of $D_p$, in other words), the large $N_m$ associated with small $D_p$ may have nothing to do with the attractiveness of the females in terms of potential mating, but may occur simply because males avoid aggressive females in early lactation. Gleason et al. (1980), however, showed that female P. leucopus were highly aggressive when their young were 20 days old, i.e., the aggression is maintained throughout lactation period. Thus, the alternative explanation is not plausible.

# CHAPTER 5 GENETIC EVIDENCE OF MULTIPLE PATERNITY

My results in Chapter 3 demonstrate that males do not provide paternal care of any sort and that they stop interacting with the females once the copulation is over. My results in Chapter 4 reveal that females close to oestrus have more males nearby than females far from oestrus. These results, especially those of Chapter 4, can be explained only in terms of males mating polygynously. I showed in Chapter 2 that females mate polyandrously in experimental enclosures. If females also mate polyandrously in the field, then I can conclude that promiscuity is a prevailing characteristic of the mating system in this species. The ultimate proof of the polyandrous mating in females lies in genetic evidence showing multiple paternity in single litters. In this chapter, I examine the genetics of multiple paternity in single litters conceived in the field and, if multiple paternity in single litters is confirmed, estimate the proportion of litters having multiple paternity in natural populations of *P. leucopus*.

## § 5.1 METHODS

The study was conducted in deciduous forests north of London, Ontario, Canada (43 N, 81 W). Adult female *P. leucopus* were sampled with grids of Longworth live traps in the summer of 1987 and 1988 and allowed to give birth to field-conceived young in the laboratory. Altogether 35 and 37 females gave birth to field-conceived young in 1987 and 1988, respectively. These animals form the data base for this study.

1. Electrophoresis of blood proteins.

Blood samples of at least 15 µl were taken by suborbital puncture from each female and her young and examined for genetic polymorphism for 5 loci, which were found to be highly polymorphic in P. leucopus populations studied by Robbins et al. (1985), using horizontal starch-gel electrophoretic procedures described in Selander et al. (1971). The 5 loci examined were 1) esterase-1, 2) haemoglobin, 3) albumin, 4) 6-phosphogluconate dehydrogenase and 5) transferrin. Except for esterase-1 (Es-1), which was highly polymorphic, the other four were either monomorphic (haemoglobin, albumin and 6-phosphogluconate dehydrogenase) or only slightly polymorphic (transferrin, with an allelic frequency of 0.984 for one allele and 0.014 for the other). Only data for Es-1 were used to detect multiple paternity in this study.

Electrophoresis was carried out by technicians in the Herpetology Laboratory of Royal Ontario Museum in 1987 and in Dr. Jim Bogart's laboratory in University of Guelph in 1988. My protocol of electrophoresis on Es-1 is the same as that of Selander et al. (1971) except for staining. My esterase stain is made of 1) 30 ml of Stock Solution A buffer (as described in Selander et al. 1971), 2) 20 ml double distilled water, 3) 25 mg Fast Blue RR (rather than Fast Garnet GBC as in Selander et al. 1971), and 4) 1 ml of stock solution containing 1 g alpha-naphthyl acetate (rather than alpha-naphthyl propionate as in Selander et al. 1971). This stain improved resolution of electromorphs. Fig. 5-1 displays a photograph of a

Fig. 5-1 Photograph of a representative gel showing polymorphism of Es-1, which is the system anodal to the hemoglobin (darkly stained and thick bands). The alleles, listed in increasing order of speed of migration of their bands, are designated A, B, C and D.

representative gel showing polymorphism of Es-1. Samples with ambiguous electromorphs were re-run to verify original scoring.

To test for possible phenotypic consistency in Es-1, twenty-eight mice of representative genotypes in 1987 were kept alive in the laboratory until 1988, and their blood scored together with those caught in 1988. Blood samples from these twenty-eight mice also served as a reference for scoring electromorphs of blood samples taken from mice caught in 1988.

2. Detection of multiple paternity.

Two methods were used in detecting multiple paternity in single litters. The first one was simply to examine whether a litter contained at least 3 different paternal alleles. The second was based on differences in number of "homogenetic" and "heterogenetic" litters between monogamy and promiscuity, a litter being homogenetic if all young in the litter share the same genotype and heterogenetic if otherwise. In Appendix 1, I demonstrated that the number of homogenetic litters would decrease, and heterogenetic litters increase, with number of males a female mates with, and explained how a one-tailed test should be done to distinguish between monogamous mating in females and polyandrous mating in females. The data needed for the method are 1) allelic and genotypic frequencies, 2) maternal genotype, and 3) litter size.

In order to test whether females mate with single males or multiple males, one needs to calculate the number of and test whether the observed number of homogenetic litters significantly less, and heterogenetic litters more, than the expected value. The method for calculating expected number of homogenetic and heterogenetic litters, given 4 alleles and 10 potential genotypes, is outlined below, following the notation in Appendix 1.

Homozygous mothers:

A homozygous female has a probability of $P_{aa}+P_{bb}+P_{cc}+P_{dd}$ of mating with a homozygous male, with the resulting litter necessarily being homogenetic; she also has a probability of $P_{ab}+P_{ac}+P_{ad}+P_{bc}+P_{bd}+P_{cd}$ of mating with a heterozygous male, with the resulting litter having a probability of $2 \cdot 0.5^n$ being homogenetic, where $n$ is litter size. Therefore, a litter of size $n$ produced by a homozygous mother has a probability of $Prob(homoF)=(P_{aa}+P_{bb}+P_{cc}+P_{dd})+2 \cdot 0.5^n \cdot (P_{ab}+P_{ac}+P_{ad}+P_{bc}+P_{bd}+P_{cd})$ being homogenetic.

Heterozygous mothers:

A heterozygous female, say AB, has a probability of $P_{aa}+P_{bb}+P_{cc}+P_{dd}$ of mating with a homozygous male, with the resulting litter having a probability of $2 \cdot 0.5^n$ being homogenetic. She also has a probability of $P_{ab}$ of mating with a male of her genotype, with the resulting litter having a probability of $2 \cdot 0.25^n+0.5^n$ being homogenetic. In addition, she has a probability of $P_{ac}+P_{ad}+P_{bc}+P_{bd}+P_{cd}$ of mating with a litter having a probability of $4 \cdot 0.25^n$ being homogenetic. Therefore, a litter of size n produced by a heterozygous female AB has a probability of

$$\text{Prob(AB female)} = 2 \cdot 0.5^n \cdot (P_{aa}+P_{bb}+P_{cc}+P_{dd}) + (2 \cdot 0.25^n+0.5^n) \cdot P_{ab} + 4 \cdot 0.25^n \cdot (P_{ac}+P_{ad}+P_{bc}+P_{bd}+P_{cd})$$

being homogenetic. Similar equations can be written out for AC, AD, BC, BD, and CD females.

By calculating the probability of each litter being homogenetic using the above equations and summing up all resulting probabilities, one obtains the number of homogenetic litters expected under monogamy. The expected number of heterogenetic litters is simply the difference between the total number of litters and the expected number of homogenetic litters.

3. Estimating frequency of litters resulting from multiple paternity.

Birdsall and Nash (1973) and Merritt and Wu (1975) introduced a method for estimating frequency of litters with multiple paternity for genetic loci of 3 different alleles and I extended the method to include loci of 4 alleles. In order to identify a litter of multiple paternity with certainty, at least 3 different paternal alleles must be identified in the litter. The probability of finding such a litter depends on 1) probability of paternal males carrying 3 or 4 different alleles of the locus in question ($Pr_1$), which in turn depends

frequency and number of males involved in multiple matings, and 2) the probability of these 3 or 4 different paternal alleles being identified in the young ($Pr_2$), which in turn depends on the maternal genotype, litter size and allelic frequency of sperms she receives, which in turn depends on number of males she mates.

The relationship between $Pr_1$, $Pr_2$, and number of males involved in multiple matings requires special attention because it is impossible to know how many males were actually involved in multiple matings in the wild or whether all multiple matings involve the same number of males. For this reason, $Pr_1$ is calculated for two extreme situations: 1) when only 2 males are involved in multiple matings (henceforth referred to as two-male case) and 2) when all males in the population are involved in multiple matings (henceforth referred to as all-male case). Both $Pr_1$ and $Pr_2$ are at a minimum in the two-male case and a maximum in the all-male case. Correspondingly, expected number of litters with multiple paternity is at a minimum in the two-male case and a maximum in the all-male case. These two estimates (minimum and maximum) provide two reference points for comparison with the observed number of litters with multiple paternity. For example, if the observed number of litters with at least 3 paternal alleles is $N_o$, and the expected number of litters with at least 3 paternal alleles is $N_{two}$ in the two-male case and $N_{all}$ in the all-male case, then the proportion of litters resulting from multiple insemination from more than one male is $N_o/N_{two}$ for the two-male case and $N_o/N_{all}$ for the all-male case. The difficulty of the method, therefore, lies in the calculation of $N_{two}$ and $N_{all}$, each of which requires separate estimation of $Pr_1$ and $Pr_2$.

Two methods can be used to calculate $Pr_1$, one deriving the probability from the observed genotypic frequency and the other from allelic frequency assuming Hardy-Weinberg equilibrium. For example, the probability of paternal males carrying 4 different alleles of Es-1 in the two-male case can be calculated either from the observed genotypic frequency as $2[(P_{ab}\times P_{cd})+(P_{ac}\times P_{bd})+(P_{ad}\times P_{bc})]$ or from allelic frequency as $24\times P_a\times P_b\times P_c\times P_d$. In this study, I used the first method because it is less affected by deviations from Hardy-Weinberg equilibrium than the second. In the all-male case, each promiscuous female will have available to her a sperm pool that has the same allelic frequency as that of the male population as a whole.

Given that a female mates with males carrying 3 or 4 different alleles ($Pr_1$), the probability that at least 3 different paternal alleles are realized in the resulting litter ($Pr_2$) can be calculated as follows.

Two-male case:

1) when the female is homozygous, then $Pr_2$ can take only 2 values, one when the female mates 2 males carrying only 3

different alleles and one when she mates two males carrying 4 different alleles. The first can be calculated by expanding the expression $(P_a+P_b+P_c)^n$ (where $P_a$ $P_b$ and $P_c$ are allelic frequency of the sperm pool contributed by the two males and equal 0.25, 0.5 and 0.25, respectively, and n is litter size) and summing up those terms which include $P_a$ $P_b$ and $P_c$. The second can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$ (where $P_a=P_b=P_c=P_d=0.25$, n=litter size) and summing up those terms which include any 3 of $P_a$ $P_b$, $P_c$ and $P_d$.

2) When the female is heterozygous, then $Pr_2$ can take 5 values; 4 values when males carry 3 different alleles and 1 value when males carry 4 different alleles. The last value can be calculated by expanding $(P_a+P_b+P_c+P_d+P_e)^n$ (where $P_a$ is the probability of young sharing the same genotype as the mother, $P_b$ and $P_c$ are the probabilities of young being homozygous for each of the alleles carried by the mother, and $P_d$ and $P_e$ is the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother) and summing up those terms that include 1) $P_a$, $P_d$ and $P_e$, or 2) $P_b$, $P_d$ and $P_e$, or 3) $P_c$, $P_d$ and $P_e$ or 4) $P_b$, $P_c$ and $P_d$, or 6) $P_b$, $P_c$ and $P_e$. The 4 values when two males carry 3 different alleles of a locus can be calculated as follows:

1) When the heterozygous female has the allele present twice in males and lacks only one allele present in males

(e.g. AB female with AB, AC males), $Pr_2$ can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$ (where $P_a$ is the probability of the offspring sharing the same genotype as the mother, $P_b$ and $P_c$ are the probabilities of an individual being homozygous for each of the alleles carried by the mother, and $P_d$ is the sum of the probabilities of an offspring carrying the allele not present in the mother) and summing up those terms that include $P_b$, $P_c$ and $P_d$.

ii) When the heterozygous female has the allele present twice in males and lacks 2 alleles present in males (e.g. AB female with AC, AD males), $Pr_2$ can be calculated by expanding $(P_a+P_b+P_c)^n$ (where $P_b$ and $P_c$ are the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother and $P_a=1-P_b-P_c$, n=litter size) and summing up those terms that include $P_a$ $P_b$ and $P_c$.

iii) When the heterozygous female lacks the allele present twice in males but possesses the other 2 paternal alleles (e.g. AB female with AC, BC males), $Pr_2$ can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$, where symbols mean the same as in i), and summing up those terms that include $P_b$, $P_c$ and $P_d$.

vi) When the heterozygous female lacks the allele present twice in males and possesses only one of the 3 paternal alleles, $Pr_2$ is the same as in ii).

All-male case:

1) When the female is homozygous, $Pr_2$ can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$ (where $P_a$ $P_b$, $P_c$ and $P_d$ are gene frequencies of the population and n=litter size) and summing up those terms that include any three of $P_a$ $P_b$, $P_c$ and $P_d$.

2) When the female is heterozygous, $Pr_2$ can be calculated by expanding $(P_a+P_b+P_c+P_d+P_e)^n$ (where $P_a$ is the probability of young sharing the same genotype as the mother, $P_b$ and $P_c$ are the probabilities of young being homozygous for each of the alleles carried by the mother, and $P_d$ and $P_e$ is the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother) and summing up those terms that include 1) $P_a$, $P_d$ and $P_e$, or 2) $P_b$, $P_d$ and $P_e$, or 3) $P_c$, $P_d$ and $P_e$ or 4) $P_b$, $P_c$ and $P_d$, or 6) $P_b$, $P_c$ and Pe.

It should be mentioned that the above method assumes the absence of a null allele or presence of it at negligible frequency. Robbins et al (1985) studied genetic polymorphism of Es-1 for 21 populations of P. leucopus over north America and no null allele was reported: the assumption of the absence of a null allele is likely justified.

## § 5.2 RESULTS

Samples of P. leucopus taken in 1987 and 1988 had the same 4 alleles and 10 potential genotypes (Table 5-1). Blood samples taken from the same mouse in 1987 and 1988 (N=28) did not show different band patterns, suggesting phenotypic consistency of Es-1 at different times in this population of *P. leucopus*. The observed genotypic frequencies fit closely to those expected under Hardy-Weinberg equilibrium (Table 5-1). The greatest discrepancy between the observed and expected genotypic frequencies occurs in young sampled in 1988 (Table 5-1), but the probability that the discrepancy being due to chance is still greater than 0.05, based on a Chi-square test of goodness-of-fit. There are some reasons that the population may indeed be in Hardy-Weinberg equilibrium. First, alleles at Es-1 locus are usually considered neutral (Singh, pers. comm.), so the effect of selection in relation to Hardy-Weinberg equilibrium may be ruled out. Second, adult females do not disperse (Wolff 1989), so the distribution of genotypic frequency in adult females should not be affected by migration. If a proportion of young were immigrants from a population with different genotypic frequencies, then genotypic frequency should differ between adult females and young. The fact that this did not happen (Table 5-1) suggests that migration can also be ruled out as a factor affecting distribution of genotypic frequencies. The last factor affecting Hardy-Weinberg equilibrium (excluding mutation, which should be trivial any way) is the breeding system. If random mating did not hold for our population, then we should expect observed genotypic frequencies to deviate from Hardy-Weinberg equilibrium, which did not occur (Table 5-1). Thus,

Table 5-1 Genotypic frequencies for Es-1 of samples taken in 1987 and 1988.

| Geno-Type | 1987 | | | | 1988 | | | |
|---|---|---|---|---|---|---|---|---|
| | Dam | | Young | | Dam | | Young | |
| | O | E | O | E | O | E | O | E |
| AA | 22 | 21.68 | 93 | 90.15 | 14 | 13.60 | 43 | 41.00 |
| AB | 3 | 4.25 | 19 | 21.06 | 10 | 12.01 | 51 | 63.89 |
| AC | 2 | 1.70 | 4 | 3.37 | 0 | 0.00 | 1 | 1.01 |
| AD | 2 | 1.70 | 5 | 9.27 | 5 | 3.79 | 25 | 16.10 |
| BB | 1 | 0.21 | 3 | 1.23 | 4 | 2.65 | 34 | 24.89 |
| BC | 0 | 0.17 | 0 | 0.39 | 0 | 0.00 | 1 | 0.78 |
| BD | 0 | 0.17 | 0 | 1.08 | 1 | 1.68 | 7 | 12.54 |
| CC | 0 | 0.03 | 0 | 0.03 | 0 | 0.00 | 0 | 0.01 |
| CD | 0 | 0.07 | 0 | 0.17 | 0 | 0.00 | 0 | 0.20 |
| DD | 0 | 0.03 | 3 | 0.24 | 0 | 0.26 | 0 | 1.58 |

Note: O -- Observed; E -- Expected.

our assumption of Hardy-Weinberg equilibrium may be justified.

The result of a close fit between the observed and expected genotypic frequencies also lends support to our assumption of the absence of a null allele for the following reason. If a null allele were present, then individuals heterozygous for this null allele and a scorable allele would have been recorded as homozygotes for that scorable allele (false homozygotes). If the frequency of the null allele was as high as 0.05, then approximately 10% of individuals in the population would have been false homozygotes. These false homozygotes, added to those true homozygotes, would have resulted in an excess of homozygotes and a deficiency of heterozygotes to upset Hardy-Weinberg equilibrium, given our sample size. The fact that no significant departure from Hardy-Weinberg equilibrium was observed in both years suggests that the null allele, if present, must be at a very low level.

I. Detection of multiple paternity in single litters.

One litter in 1987 and 6 litters in 1988 contained 23 different paternal alleles (Table 5-2), which implies that they resulted from multiple insemination by more than one male.

Of 3[illegible] litters in 1987 and 33 litters in 1988 that had at least 2 scorable young, the observed number of homogenetic litters were smaller and heterogenetic litters greater than those expected under the assumption of monogamy (Table 5-3). A one-tailed significance test (Appendix 1), with its null hypothesis stating that observed heterogenetic litters is no

Table 5-2. Litters with multiple paternity in 1987 and 1988.

| Year | Maternal genotype | Offspring genotype |
|---|---|---|
| 1987 | AA | AB,AA,AD. |
| 1988 | AA | AA,AB,AB,AD,AD |
| | AA | AA,AB,AB,AB,AC,AD |
| | AA | AA,AB,AB,AB,AD |
| | AA | AA,AA,AA,AB,AB,AD |
| | AA | AA,AA,AB,AB,AD |
| | AA | AA,AB,AB,AD,AD,AD |

2

OF/DE

2

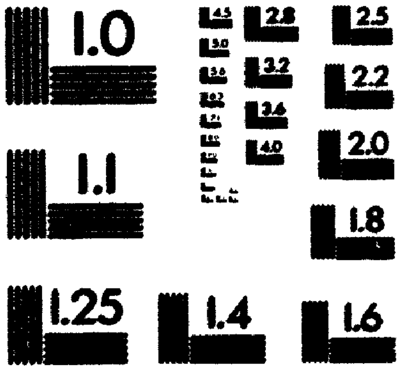


Table 5-3. Observed number of "homogenetic" litters (all young in the litter having the same genotype) and "heterogenetic" litters compared with expected values calculated with the assumption of monogamy.

| year | litter | observed | expected |
|---|---|---|---|
| 1987 | "heterogenetic | 15 | 10 |
| | "homogenetic" | 15 | 20 |
| 1988 | "heterogenetic" | 31 | 23 |
| | "homogenetic" | 2 | 10 |

more than the expected, was done by summing up the probabilities of obtaining the observed number of homogenetic and heterogenetic litters and all other frequencies representing a greater deviation from expectation. The null hypothesis was rejected in both years (P = 0.0434 in 1987 and 0.0008 in 1988). Thus multiple paternity in single litters is evident.

II. Estimating frequency of litters due to multiple paternity.

1) Two-male case. The probability of a female encountering 2 mates carrying 3 or 4 different alleles ($Pr_1$) was calculated separately for 1987 and 1988 (Table 5-4, 5-5). Given $Pr_1$, the probability of a female producing a litter with at least 3 different paternal alleles present ($Pr_2$) was calculated for different combinations of maternal genotype and litter size (Table 5-6). I then calculated the probability that mothers of different genotypes and litter sizes produced litters with at least 3 different paternal alleles. I will use mothers AA and AB of 1987, each producing a litter of 5 to illustrate the actual calculation. For mother AA, the probability of her mating 2 males carrying 3 different alleles was 0.06440 (Table 5-5) and, given such a mating, the probability of her having a litter carrying all 3 paternal alleles was 0.527344 (Table 5-6). The product of the 2 probabilities was 0.03396. The female also had a probability of 0.00153 (Table 5-5) of mating 2 males carrying 4 different alleles and, given such a mating, the probability of her

Table 5-4. Probability of a female encountering different sperm pool given that only two males are involved in multiple matings and contribute equally to the sperm pool.

| Paternal | Probability | |
|---|---|---|
| alleles | 1997 | 1988 |
| 2A.B.C | 0.015603 | 0.005517 |
| 2A,B.D | 0.033808 | 0.113097 |
| 2A.C.D | 0.006761 | 0.001300 |
| 2B.A.C | 0.001766 | 0.004017 |
| 2B.A.D | 0.003827 | 0.082338 |
| 2B.C.D | 0.000087 | 0.000689 |
| 2C.A.B | 0.000353 | 0.000046 |
| 2C,A,D | 0.000153 | 0.000011 |
| 2C.B.D | 0.000017 | 0.000033 |
| 2D,A,B | 0.001553 | 0.019402 |
| 2D.A.C | 0.000322 | 0.003223 |
| 2D.B.C | 0.000036 | 0.000162 |
| | | |
| A.B.C.D | 0.001531 | 0.001993 |

Table 5-5. Probability of females of different genotypes obtaining sperms of different combinations of at least 3 different paternal alleles, assuming that only 2 males are involved in multiple matings.

| Maternal genotype* | Paternal alleles | Special case** | Probability | |
|---|---|---|---|---|
| | | | 1987 | 1988 |
| AA.BB | 3 | | 0.06440 | 0.22687 |
| | 4 | | 0.00153 | 0.00189 |
| AB | 3 | 1 | 0.05501 | 0.20496 |
| | | 2 | 0.00695 | 0.00198 |
| | | 3 | 0.00201 | 0.01944 |
| | | 4 | 0.00054 | 0.00040 |
| | 4 | | 0.00153 | 0.00189 |
| AC | 3 | 1 | 0.02297 | 0.00688 |
| | | 2 | 0.03383 | 0.11310 |
| | | 3 | 0.00210 | 0.00424 |
| | | 4 | 0.00561 | 0.10280 |
| | 4 | | 0.00153 | 0.00189 |
| AD | 3 | 1 | 0.04266 | 0.13404 |
| | | 2 | 0.01564 | 0.00568 |

Table 5-5 (continued)

| Maternal genotype* | Paternal alleles | Special case** | Probability 1987 | Probability 1988 |
|---|---|---|---|---|
| | | 3 | 0.00408 | 0.08234 |
| | | 4 | 0.00222 | 0.00476 |
| | 4 | | 0.00153 | 0.00189 |

*Although there are 10 possible genotypes with 4 alleles, only genotypes AA, AB, AC, AD and BB were found in mothers.

**Special case: 1--The female has the allele present twice in males and lacks only one allele present in males; 2--The female has the allele present twice in males and lacks 2 other alleles present in males; 3--The female lacks the allele present twice in males but possesses the other 2 paternal alleles; 4--The female lacks the allele present twice in males and possesses only one of the 3 alleles in males.

Table 5-6. Probability of a female producing a litter with at least 3 different paternal alleles present, given maternal genotype, number of paternal alleles, and litter size, assuming only 2 males are involved in the multiple mating.

| Maternal genotype | Paternal alleles | Special case | Litter size | Probability |
|---|---|---|---|---|
| homoz. | 3 | | 3 | 0.18750 |
| | | | 4 | 0.375 |
| | | | 5 | 0.52734 |
| | | | 6 | 0.64453 |
| | | | 7 | 0.73315 |
| | 4 | | 3 | 0.375 |
| | | | 4 | 0.65625 |
| | | | 5 | 0.82031 |
| | | | 6 | 0.90820 |
| | | | 7 | 0.95361 |
| heteroz. | 3 | 1 | 3 | 0.04688 |
| | | | 4 | 0.12988 |
| | | | 5 | 0.22705 |
| | | | 6 | 0.32730 |
| | | | 7 | 0.42161 |
| | | 2 | 3 | 0.18750 |
| | | | 4 | 0.375 |
| | | | 5 | 0.52734 |

Table 5-6 (continued)

| Maternal genotype | Paternal alleles | Special case | Litter size | Probability |
|---|---|---|---|---|
| | | | 6 | 0.64453 |
| | | | 7 | 0.73315 |
| | | 3 | 3 | 0.04688 |
| | | | 4 | 0.11719 |
| | | | 5 | 0.19409 |
| | | | 6 | 0.27008 |
| | | | 7 | 0.34230 |
| | | 4 | 3 | 0.18750 |
| | | | 4 | 0.375 |
| | | | 5 | 0.52734 |
| | | | 6 | 0.64453 |
| | | | 7 | 0.73315 |
| | 4 | | 3 | 0.23438 |
| | | | 4 | 0.46875 |
| | | | 5 | 0.64819 |
| | | | 6 | 0.77271 |
| | | | 7 | 0.85516 |

having a litter with at least 3 different paternal alleles present was 0.820311 (Table 5-6). The product of the 2 probabilities is 0.00126. The sum of these 2 products (=0.03522) is the probability of an AA mother, in the two-male case, producing a litter of 5 that contains at least 3 different paternal alleles. The calculation for the AB mother is much more complicated (Table 5-7) and the probability that she has a litter containing at least 3 different paternal alleles is equal to 0.0178217, which is the sum of the last column, i.e., $Pr_1/Pr_2$, in Table 5-7. Such calculation was carried out for each female with at least 3 young in 1987 (N=29) and 1988 (N=32). The expected number of litters that contain at least 3 different paternal alleles was 3.6 (0.7 in 1987 and 2.9 in 1988) in the two-male case. The observed number of litters that contain at least 3 different paternal alleles was 7 (1 in 1987 and 6 in 1988). As 7 out of a sample of 61 (= 29 + 32) has a lower limit of 2.6 at 0.95 confidence interval (Sokal and Rohlf, 1981), I conclude that, given the two-male case, at least 72% (= 2.6/3.6) of litters were sired by multiple fathers.

2). All-male case. $Pr_1$, the probability of a female mating with males carrying at least 3 different alleles, is now equal to 1, and the allelic frequency of sperms a female received is the same as the allelic frequency of the population. $Pr_2$, the probability of a female producing a litter with at least 3 different paternal alleles present, given $Pr_1$ and gametic frequency, depends only on maternal

Table 5-7. Calculation of the probability of a litter having at least 3 different paternal alleles given an AB mother in 1987 with a litter of 5.

| Paternal alleles | Special case | Pr1 | Pr2 | Pr1*Pr2 |
|---|---|---|---|---|
| 3 | 1 | 0.05501 | 0.227051 | 0.0124901 |
| | 2 | 0.00695 | 0.527344 | 0.0036650 |
| | 3 | 0.00201 | 0.194092 | 0.0003901 |
| | 4 | 0.00054 | 0.527344 | 0.0002848 |
| 4 | | 0.00153 | 0.648193 | 0.0009917 |

genotype and litter size (Table 5-8). If a homozygous female produced a litter of 5 in 1983, then the probability of this litter containing at least 3 different paternal alleles was 0.338691; if a heterozygous female such as AB produced a litter of 5 in 1988. then the litter had only a probability of 0.163905 of containing at least 3 different paternal alleles (Table 5-8). The expected number of litters containing at least 3 paternal alleles was 2.0 in 1987 and 8.3 in 1988, as calculated from 29 mothers in 1987 and 32 mothers in 1988, with each of the mothers having at least 3 scorable young. Thus the frequency of litters due to multiple insemination involving all males was 50% (=1/2.0) in 1987 and 72% (=6/8.3) in 1988. Assuming that the degree of multiple paternity does not change over years, then the average of the frequency is 0.68 (=7/10.3). Because 7 out of a sample size of 61 (=29+32) litters had a lower limit of 2.6 at 0.95 confidence interval (Sokal and Rohlf, 1981), the lower limit of frequency of litters due to multiple insemination involving all males was 25% (=2.6/10.3). The real value was almost certainly larger than 25% because it is virtually impossible for a female to mate with all males in the population.

## § 5.3 DISCUSSION

My finding of multiple paternity in single litters confirms my prediction, based on results of previous chapters, that females in wild populations of <u>P. leucopus</u> are promiscuous. I can now state with confidence that Myton's (1974) observation of social groups with one adult female and

Table 5-8. Probability of a litter with at least 3 different paternal alleles when all males are involved in multiple matings.

| Maternal genotype | litter size | Probability<br>1987 | <br>1988 |
|---|---|---|---|
| AA,BB | 3 | 0.033733 | 0.120397 |
| | 4 | 0.065935 | 0.238503 |
| | 5 | 0.101480 | 0.338691 |
| | 6 | 0.140132 | 0.421466 |
| | 7 | 0.180779 | 0.490142 |
| AB | 3 | 0.011778 | 0.032046 |
| | 4 | 0.030048 | 0.090062 |
| | 5 | 0.053674 | 0.163905 |
| | 6 | 0.080859 | 0.244084 |
| | 7 | 0.110319 | 0.323934 |
| AC | 3 | 0.023795 | N/A |
| | 4 | 0.050067 | N/A |
| | 5 | 0.080251 | N/A |
| | 6 | 0.113502 | N/A |
| | 7 | 0.148709 | N/A |
| AD | 3 | 0.015707 | 0.035522 |
| | 4 | 0.036350 | 0.087701 |
| | 5 | 0.061501 | 0.144921 |
| | 6 | 0.089662 | 0.201102 |
| | 7 | 0.119617 | 0.253687 |

Note: N/A--Not applicable.

several adult males is due to adult males clumping temporarily around an adult female near oestrus. Mineau and Madison's (1977) observation of "pair activity" is either due to extremely low density or because only one male and one female out of a group of mice were radio-tracked. The paternal care documented by Horner (1947), McCarty and Southwick (1977) and Hartung and Dewsbury (1979) must have resulted from confined caging conditions. The mating system in wild populations of P. leucopus appears to involve simultaneous polyandry in females and serial polygyny in males. At least 25% (the upper limit being 100%) of litters conceived under natural conditions result from multiple insemination involving more than one male.

The fact that females are promiscuous implies that males are also promiscuous, because, as I argued in chapter 4, the sex ratio of adults is not male-biased in my population. This is consistent with my findings (Chapter 4) that a female near oestrus has more adult males around her than a female far from oestrus. There are several possible reasons why males should be promiscuous. For purpose of illustration, let me start with a monogamous population with males providing paternal care and examine if males that mate promiscuously can increase in frequency in the population. Whether promiscuity will be favoured will depend on the fitness increment derived from paternal care relative to that derived from remating. It is thus worthwhile to consider factors that affect the fitness increment derived from paternal care and remating. First, the probability of remating is high in wild populations of P. leucopus as there are oestrous females throughout the entire breeding season. Second, high natural mortality in adult males would favour females that can rear the young single-handedly, leading to a reduced importance of paternal care. This would also favour males deserting their mates and seeking extra mates. Third, monogamy with paternal care implies commitment of a male to a specific female. Unfortunate for this mating strategy, reproductive success of females in P. leucopus (Rintamaa et al. 1976) varies widely, with some females producing no young in the entire breeding season and some producing as many as 8 litters (Rintamaa et al., 1976). If a male bet his reproductive success on a single female, then his reproductive success will fluctuate more widely than that of a promiscuous male. For example, the variance of reproductive success of promiscuous males mating 4 females is half (= $1/\sqrt{4}$) of that of monogamous males, other things being equal. This within-generation difference in variance of fitness may result in inter-generation difference in variance of fitness in a small population. It can be shown numerically that, in a small population, a genotype is at a selective disadvantage if its fitness fluctuate more widely over generations than the competing genotype (Gillespie 1977). Because all these three reasons appear to reduce the benefit of paternal care and increase the benefit of remating, it is not surprising that males should be promiscuous.

While it is not difficult to see the evolutionary advantage of promiscuity in male P. leucopus, it is by no means easy to understand why females should mate promiscuously. One possible explanation involves pseudopregnancy in the species. Dewsbury (1984a, 1984b) found that male deer mice lose much of their inseminating power in terms of sperm output after a few consecutive copulations, but their vigour in copulation can stimulate pseudopregnancy (Conaway 1971). The duration of pseudopregnancy in Cricetidae is about two weeks (Conaway, 1971, Dewsbury, 1984a), which is a significant portion of a limited breeding season. As Conaway (1971) pointed out, a nonpregnant cycle in short-lived rodents appear to be a pathological luxury that should not be tolerated by natural selection. One strategy for females against pseudopregnancy would be to mate with more than one male so as to increase the likelihood of mating with at least one "fresh" male. My confidence in this explanation was somehow weakened by my own observation. First, in my experiment on paternal care (Chapter 3), males always followed the pre-partum females closely for severally days until parturition, and, during the pre-partum period, the male and the female had synchronized activity patterns. Such males are unlikely to become sperm-depleted unless there are oestrous females close by. Of all females I caught in the field, there is only one incidence when two neighbouring females had similar parturition dates (two days apart). Based on these observations, it may be tenuous to argue that the risk of mating with a sperm-depleted male qualify as significant selection pressure for female promiscuity.

Another explanation for female promiscuity involves the assumption that repeated ejaculations by one male result in reduced proportion of normal sperm (or increased proportion of abnormal sperm), leading to an overall decrease in semen quality (Gibson and Jewell 1982). This has been demonstrated in sheep (Tomkins and Bryant 1976; Simpson and Edey 1979; both cited in Gibson and Jewell 1982) and presumably may occur in other animals as well. One strategy against poor semen quality caused by repeated ejaculations would be to sample the first few ejaculations from many males instead of accepting many ejaculations from a single male. Some recent studies have shown that females in many rodent species require sufficient vaginal stimulation for pregnancy initiation (Dewsbury 1984c; Huck et al. 1988). This may serve as a proximate mechanism against pseudopregnancy and poor sperm quality. In the golden hamster, Mesocricetus auratus (Huck et al. 1988), only 25% of females become pregnant when mating with a single male late in receptive period, but the proportion increases to 36% when two vasectomized males were added to provide additional mating stimulation.

It is likely that there will be more interference among males as the number of males participating in multiple-male copulation increases. Interference among males in copulation may explain why postpartum copulation in P. leucopus happened in the open rather than in nest boxes and why the female was on the run during her receptive period (Chapter 2). If the female stayed in her burrow with several males cramped around her, interference among males may result in no ejaculation at all; or if the female remains motionless in the open, then males will be crowded around her as well, resulting in high degree of interference. Females therefore should be on the run all the time in the open during her receptive period.

# CHAPTER 6 GENERAL DISCUSSION

I started with a verbal model predicting seasonal variation in mating system of P. leucopus. Some assumptions of the model were later proved to be unrealistic. For example, I assumed that females were continuously aggressive towards strange males from late pregnancy to early lactation, a period encompassing postpartum oestrus. Results in Chapter 2 showed that this was false because females are not aggressive to any males during her postpartum oestrus. The model also assumed that animals had the option of forming stable pair-bond, but high mortality may have made this option unlikely. I may have also been misled by Hill's (1972) experiment on development of thermo-regulation in juvenile P. leucopus and overestimated the importance of paternal care in terms of huddling by the father with young. The nest of the white-footed mice in their natural habitats may not be as exposed as those in Hill's experiment. In addition, I have never seen a lactating female eating outside nest boxes, although males ate outside quite often. When a female found a piece of food, she brought it back to a nest box. Presumably, all her eating was done in her nest and this would have reduced her time away from young.

In spite of these defects in my original verbal model, I have collect convincing evidence to show that females do not reject strange males and accept familiar males as mates, and they mate promiscuously when there are more than one male available. Adult males in the wild adjust their spatial position according to breeding status of females, with females close to oestrous having more adult males in her vicinity than females far from oestrous. Multiple paternity in single litters are common (25-100%) in the wild. Multiple-male matings result in uncertainty of paternity and this may explain the lack of paternal care in the species. In short, promiscuity is a prevailing character in the mating system of P. leucopus.

What remains unanswered is whether there are still some males that provide paternal care and some females that do not mate promiscuously. Although all my experimental females mated promiscuously (Chapter 2) and none of the experimental males exhibited paternal care (Chapter 3), I cannot exclude the possibility that a non-promiscuous female (i.e., a female mating with only one male during one oestrus) or a paternal male may show up if I continue my experimental observation indefinitely. Similarly, my finding that adult males adjust their position according to reproductive status of females cannot be generalized to all males either, because only a fraction of males that adjust their position in relation to females' reproductive status would be sufficient to yield the results I presented in Chapter 4. Finally, the proportion of litters resulting from multiple insemination by more than one male has a lower limit of 25% (Chapter 5), i.e., there is a probability that some litters are not the product of multiple matings. Based on these results alone, I cannot exclude the possibility that different males and different females may have different mating strategies in natural populations of P.

*leucopus*.

Different mating strategies coexisting in the same population have been observed in many species of diverse taxonomic groups, e.g., in the red deer, *Cervus elachus* (Clutton-Brock 1982), the elephant seals, *Mirounga angustirostris* (Le Boeuf 1974), the bullfrog, *Rana catesbiana* (Howard 1978), the bee, *Centris pallida* (Alcock *et al.* 1977), the field cricket, *Gryllus integer* (Cade 1979), the fig wasps, *Idarnes spp.* (Hamilton 1979), and the salmon, *Oncorhynchus kisutch* (Gross 1985). Indeed, as Krebs and Davies (1987) pointed out, there is little reason to believe that animals of the same species and the same sex should always have the same mating strategy. Mating system of animal populations may not be one of monogamy, polygyny, polyandry, and promiscuity, but may instead be a combination of some of them.

Recognizing the fact that empirical data will never be able to exclude the possibility that some males are paternal and some females are non-promiscuous in *P. leucopus*, unless sample sizes were infinite, I believe it more appropriate to examine this possibility with a modelling approach. Let me first outline some major parameters that may affect the fitness of males and females so that assumptions involved in the model will be explicit:

$N_p$ - mean number of matings a paternal male can have during one breeding season.

$N_{np}$ - means number of matings a non-paternal male can have during a breeding season; $N_{np} > N_p$.

$N_l$ - number of litters a female can produce during the breeding season.

L - litter size.

S - Survival of young.

$S_p$ - survival increment of young with paternal care.

R - mean genetic relatedness of young to males participating in a copulation episode. R = 0.5/n, where n = number of males a promiscuous female mates with.

$Q_m$ - semen quality for females with multiple-male matings;

$Q_p$ - semen quality for females when mating with a single paternal male;

$Q_{np}$ - semen quality for females when mating with a single non-paternal male.

$S_{Qm}$ - survival increment of young with semen quality $Q_m$ relative to that with semen quality $Q_{np}$; $S_{Qm} \geq 0$.

$S_{Qp}$ - survival increment of young with semen quality $Q_p$ relative to that with semen quality $Q_{np}$; $S_{Qp} \geq 0$. If the benefit of multiple-male matings is only for prevention of pseudopregnancy, then $S_{Qp} = S_{Qm}$; if multiple-male matings are also for increase sperm quality and variability (Parker 1984), then $S_{Qp} < S_{Qm}$.

$P_1$ - frequency of paternal males in the population.

$Q_1$ - frequency of non-paternal males; $P_1 + Q_1 = 1$.

$P_2$ - frequency of promiscuous females in the population.

$Q_2$ - frequency of non-promiscuous females; $P_2 + Q_2 = 1$.

Finally, assume that a mating strategy, i.e., being paternal or not, is genetically determined.

Given these parameters and conditions, the fitness of promiscuous and non-promiscuous females is as follows:

1) Fitness of a non-promiscuous female ($W_{mf}$). A non-promiscuous female has a probability of $P_1$ of mating with a paternal male, with the resulting fitness of $N_1 \cdot L \cdot S(1+S_p+S_{Qp}) \cdot 0.5$, and $Q_1$ of mating with a non-paternal male, with the resulting fitness of $N_1 \cdot L \cdot S \cdot 0.5$. Thus

$$W_{mf} = P_1 \cdot N_1 \cdot L \cdot S(1+S_p+S_{Qp}) \cdot 0.5 + Q_1 \cdot N_1 \cdot L \cdot S \cdot 0.5.$$

2) Fitness of a promiscuous female ($W_{pf}$). Let n be the number of males a promiscuous female mates with. Her fitness is $N_1 \cdot L \cdot S \cdot (1+S_{Qm}) \cdot 0.5$ when all her mates are non-paternal, the probability of which being $Q_1^n$, and $N_1 \cdot L \cdot S(1+S_p+S_{Qm}) \cdot 0.5$ when at least one of her mates is paternal, the probability of which being $1-Q_1^n$. Thus

$W_{pf} = Q_1^n \cdot N_1 \cdot L \cdot S \cdot (1+S_{Om}) \cdot 0.5 + (1-Q_1^n) \cdot N_1 \cdot L \cdot S(1+S_p+S_{Om}) \cdot 0.5$.

It is apparent that $W_{mf}$ will never be greater than $W_{pf}$, regardless the frequency of paternal males and the importance of paternal care. If there is no paternal males ($P_1 = 0$), then females should mate promiscuously to get better sperm; if all males are paternal ($P_1 = 1$), then females should mate promiscuously to improve sperm quality because, with $P_1=1$, paternal care is guaranteed. If $P_1$ is between 0 and 1, then females should mate promiscuously as well to 1) improve sperm quality, and 2) to increase her chance of mating with at least one paternal male so as to get paternal care for her young. Females should also mate promiscuously if paternal care is unimportant.

Given that females are promiscuous, the fitness of a non-paternal male ($W_{np}$) is of two-fold. First, there is a probability of $Q_1^n$, where n being the number of males a female mates with, that all his co-sires are non-paternal. In this case his fitness from the mating will be $L \cdot S \cdot (1+S_{Om}) \cdot 0.5/n$. Secondly, there is a probability of $(1-Q_1^n)$ that at least one of his co-sires is paternal, i.e., the resulting young will receive paternal care, and in this case his fitness from the mating is $L \cdot S \cdot (1+S_p+S_{Om}) \cdot 0.5/n$. The fitness of a paternal male ($W_p$) is $N_p \cdot L \cdot S \cdot (1+S_p+S_{Op}) \cdot 0.5/n$. The frequency of paternal males will increase if $W_p > W_{np}$, which requires the condition that

$$N_p \cdot (1+S_p+S_{Qp}) > N_{np}[Q_1{}^n \cdot (1+S_{Qm}) + (1-Q_1{}^n) \cdot (1+S_p+S_{Qm})]. \quad [1]$$

Paternal and non-paternal males may co-exist if the terms on both sides of [1] are equal.

Clearly, if all males are paternal ($P_1=1$, $Q_1=0$), then the term on the left side of [1] will always be smaller than the right. In other words, a population of paternal males are vulnerable to invasion of non-paternal males as the benefit of cuckoldry increases with increasing frequency of paternal males. When there is no paternal male ($P_1=0$, $Q_1=1$), then [1] reduces to

$$\frac{(1+S_p+S_{Qp})}{(1+S_{Qm})} \qquad \frac{N_{np}}{N_p} \qquad [2]$$

which is the condition for paternal mutants to invade a non-paternal population. Average number of matings per male in a non-paternal population ($N_{np}$) with a 1:1 sex ratio equals the mean number of oestrus a female experiences during a breeding season multiplied by the number of males involved in each multiple-male mating. If a female experiences oestrus three times and produce three litters during one breeding season, and each mating involves 3 males, then $N_{np}$ would be 9 and $N_p$ would 3. This requires the paternal male to at least triple the survival of the young receiving his care in order to invade the non-paternal population. I believe that paternal care of such great effect is very unlikely in P. leucopus in which males neither feed the young nor defend territories. Thus, if the assumptions involved in the model are realistic in P. leucopus, then females should be promiscuous and males should be non-paternal. and there should be no alternative mating strategies. To date, there has been no evidence against any assumptions of this model.

As an extension of the model. suppose that the mating strategy of males is flexible in that a male will be paternal only when a female mates with him alone, otherwise he will be non-paternal. Clearly. males of this flexible mating strategy will be favoured by natural selection over both pure paternal and pure non-paternal males. Females now have to mate either monogamously to take advantage of paternal care, or commit adultery by mating different males at different locations or different times to take advantage of both paternal care and multiple-male mating. I have shown evidence of honest promiscuity in females (Chapter 2) who not only mate with multiple males. but also mate with them at the same time and location. This suggests that either males of the flexible mating strategy do not exist. or paternal care is unimportant. in which case females will be promiscuous and males of this flexible mating strategy will be the same as non-paternals.

It is perhaps beneficial to compare P. leucopus with other species where alternative mating strategies were observed. In the red deer. Cervus elaphus (Clutton-Brock

1982), the elephant seals, *Mirounga angustirostris* (Le Boeuf 1974), and the bullfrog, *Rana catesbiana* (Howard 1978), sexually mature males differ much in body size due to age differences. The size difference results in differences in fighting and defending females or territories. Small, young males are not strong enough to defend a territory or a harem. So they make the best out of a bad situation by sneaking some copulations from females in the territory of a large, old male. In the bee, *Centris pallida* (Alcock *et al.* 1977), males differ in size due to differences in nutritional status at early age. Large males dig up virgin females, defend them against other males, and copulate with them, while small males, who could not defend females even if they could dig them up, predictably do no digging, but pursue airborne females who were not mated by diggers. In the fig wasps, *Idarnes spp.* (Hamilton 1979), and the salmon, *Oncorhynchus kisutch* (Gross 1985), difference in fighting and defending ability in males results from dramatic dimorphism in males, and different mating strategies were likely maintained by frequency dependent selection. All these examples involve some males fighting and defending females or territories essential for successful breeding and other males, who are less capable of fighting and defending, adopting alternative mating strategies. In *P. leucopus*, however, I observed no male defending a female during her oestrus against other males. Males are unlikely to hold a territory for one or more females against other females either, because all my experimental males ran away from aggressive females.

I found Cade's (1979) study on the field cricket, *Gryllus integer* most relevant to *P. leucopus* in terms of alternative mating strategies. Some male crickets call and others keep silent. Callers are more successful in attracting females and mating, but also attract more parasites and suffer higher mortality than non-callers. Some *P. leucopus* populations are heavily infested by larvae of the bot fly, *Cuterebra fontinella* (Wecker 1962; Whitaker 1968; Timm and Cook 1979; Catts 1982). I have seen bots so large on the scrotal region of the white-footed mice that the mice have to drag themselves forward as their hindfeet can barely touch the ground. I believe that the infested mice must have suffered a reduction in their fitness. The infestation is by physical contact of mice with bot fly eggs laid in environs frequented by the host, and greater mobility of the host implies greater chance of infestation. In other words, male *P. leucopus* that visit and, presumably mate with, more females will be more likely infested and suffer higher mortality, i.e., greater benefit associated with greater cost. Whether infestation by larvae of the bot fly affect the mating strategy of the white-footed mice may be a promising area for future study.

# Appendix 1

## Demonstration That "Homogenetic" Litters Will Decrease, and "Heterogenetic" Litters Increase, with Number of Males a Female Mates with.

Suppose a population with a polymorphic locus of 2 alleles, A and B, with corresponding allelic frequency $P_a$ and $P_b$. Denote genotype frequencies $P_{aa}$, $P_{ab}$, and $P_{bb}$. With monogamy, expected genotype frequency of young in a litter can be calculated given parental genotypes. For example, if two AA homozygotes mate, all young in the resulting litter will necessarily have the same genotype of AA. I define litters with all young sharing the same genotype as homogenetic litters. If an AB male mates an AB female, then there are 3 possible genotypes in the resulting litter, AA, AB and BB, with corresponding probabilities 0.25, 0.5 and 0.25, respectively. Litters with young having different genotypes are defined as heterogenetic litters. I will show below statistically that homogenetic litters will decrease, and heterogenetic litters increase, with the number of males each female mates with. For an intuitive understanding, one can imagine that a litter will necessarily be homogenetic if it results from a homozygous female mating with either an AA or a BB male, but a litter will have a probability of being heterogenetic if the same female mates with both an AA male and a BB male.

<u>Females each mating with only one male:</u>

When a female is homozygous, then the probability of her litter being homogenetic is 1 if she mates with a homozygous male, and equal to $2\times0.5^n$ if she mates with a heterozygous male. So the probability of a homozygous female i having a homogenetic litter can be expressed as

$$[1]\quad \text{Prob(homoF)}_i = (P_{aa}+P_{bb}) + 2\times P_{ab}\times0.5^n,$$

where n is litter size.

If a female is heterozygous, then the probability of her litter being homogenetic is $2\times0.5^n$ when she mates with a homozygous male, and $(2\times0.25^n+0.5^n)$ when she mates with a heterozygous male. Thus, the probability of a heterozygous female j having a homogenetic litter is

$$[2]\quad \text{Prob(heteroF)}_j = (P_{aa}+P_{bb})\times2\times0.5^n + P_{ab}\times(2\times0.25^n + 0.5^n).$$

The expected total number of homogenetic litters can be expressed as

$$[3]\quad \text{N(homogenetic)} = \sum_{i=1}^{N} \text{Prob(homoF)}_i + \sum_{j=1}^{M} \text{Prob(heteroF)}_j,$$

where N = number of homozygous females and M = number of heterozygous females. The expected number of heterogenetic litters is simply the difference between total number of litters and N(homogenetic).

<u>Females each mating with two males:</u>

With 2 alleles and 3 genotypes, 9 different combinations of 2 males are possible. These 9 combinations can be grouped into 3 paternal categories: 1) two males of the same homozygotes, i.e., only one type of sperm is contributed (Paternal category 1 in Table A1), 2) one homozygous male and one heterozygous male (Paternal category 2 in Table A1), contributing two different types of sperm in the ratio of 3:1, and 3) two heterozygous males, contributing 2 types of sperm in the ratio of 1:1 (Paternal category 3 in Table A1). The probability of a female of a certain genotype producing a homogenetic litter, therefore, equals the product of two probabilities: probability of the female encountering different combinations of two males ($Prob_1$ in Table A1) and probability of the female producing a homogenetic litter given $Prob_1$ and litter size ($Prob_2$ in Table A1). Using the same symbols as before,

[4]

$$Prob(HomoF)_i=(P_{aa}^2+P_{bb}^2)\times1+[2\times(P_{aa}\times P_{ab}+P_{ab}\times P_{bb})]\times(0.75^n+0.25^n)$$

$$+[P_{ab}^2+2\times(P_{aa}\times P_{bb})]\times(2\times0.5^n).$$

where $P_{aa}$, $P_{ab}$, $P_{bb}$ are genotype frequencies and n is litter size;

[5] $$Prob(HeteroF)_j=(P_{aa}^2+P_{bb}^2)\times(2\times0.5^n)+[2\times(P_{aa}\times P_{ab}+P_{ab}\times P_{bb})]\times$$

$$(0.375^n+0.5^n+0.125^n)+[P_{ab}^2+2\times(P_{aa}\times P_{bb})]\times(2\times0.25^n+0.5^n).$$

[6] $$N(homogenetic)=\sum_{i=1}^{N} Prob(homoF)_i+\sum_{j=1}^{M} Prob(heteroF)_j.$$

### Females each mating with all males:

With all males involved in multiple matings, the

Table A1. Calculation of expected number of homogenetic litters when two males are involved in multiple matings.

| Maternal[1] genotype | Paternal category | $Prob_1$[2] | $Prob_2$[3] |
|---|---|---|---|
| Homo. | 1 | $P_{aa}^2+P_{bb}^2$ | 1 |
| | 2 | $2\times(P_{aa}\times P_{ab}+P_{ab}\times P_{bb})$ | $0.75^n+0.25^n$ |
| | 3 | $P_{ab}^2+2\times(P_{aa}\times P_{bb})$ | $2\times0.5^n$ |
| Hetero. | 1 | $P_{aa}^2+P_{bb}^2$ | $2\times0.5^n$ |
| | 2 | $2\times(P_{aa}\times P_{ab}+P_{ab}\times P_{bb})$ | $0.375^n+0.5^n+0.125^n$ |
| | 3 | $P_{ab}^2+2\times(P_{aa}\times P_{bb})$ | $2\times0.25^n+0.5^n$ |

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1. Homo.--homozygote: Hetero.--heterozygote.

2. $Prob_1$--Probability of different combinations of males.

3. $Prob_2$--Probability of a resulting litter being homogenetic given maternal genotype, specific combination of males and litter size.

frequency of allele A and B in sperm is the same as allelic frequencies of the parental population, i.e. $P_a$ and $P_b$. Thus, the probability of a homozygous mother producing a homogenetic litter is

[7] $\text{Prob(HomoF)} = P_a^n + P_b^n$,

where n is litter size; and the probability of a heterozygous mother producing a homogenetic litter is

[8] $\text{Prob(HeteroF)} = (P_a/2)^n + [(P_a+P_b)/2]^n + (P_b/2)^n$.

The expected total number of homogenetic litters can be expressed as

[9] $$N(\text{homogenetic}) = \sum_{i=1}^{N} \text{Prob(homoF)}_i + \sum_{j=1}^{M} \text{Prob(heteroF)}_j .$$

Clearly, the number of homogenetic litters decreases with number of males a female mates with, being the largest when each female mates with a single male and smallest when the female mates with all males in the population.

A numerical example:

In a population with allelic frequencies $P_a$=0.4 and $P_b$=0.6, the probability of females producing a homogenetic litters is calculated in Table A2 for different maternal genotypes and litter sizes, using the equations presented above. For example, a homozygous female with a litter of 4 has a probability of 0.58, 0.36 and 0.16, of producing a homogenetic litter if she mates with one, two or all males, respectively. A heterozygous female with a litter of 5 has a

Table A2. Probability of females producing a homogenetic litter.

| Maternal genotype | Number of mates | Litter size | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 7 |
| Homozygotes | 1 | 0.76 | 0.64 | 0.58 | 0.55 | 0.54 | 0.53 |
| | 2 | 0.64 | 0.46 | 0.36 | 0.30 | 0.26 | 0.23 |
| | all | 0.52 | 0.28 | 0.16 | 0.09 | 0.05 | 0.03 |
| Heterozygote | 1 | 0.44 | 0.21 | 0.10 | 0.05 | 0.02 | 0.01 |
| | 2 | 0.41 | 0.18 | 0.09 | 0.04 | 0.02 | 0.01 |
| | all | 0.38 | 0.16 | 0.07 | 0.03 | 0.02 | 0.01 |

probability of 0.05, 0.04, and 0.03 of producing a homogenetic litter if she mates with one, two or all males, respectively. Summing up all probabilities for all females mating with one male, one obtains the expected number of homogenetic litters with a monogamous mating system. Summing up all probabilities for all females mating with two males, one obtains the expected number of homogenetic litters when all females are promiscuous and each female mates with two males. In the same way, one can obtain the expected number of homogenetic litters when all females are promiscuous and each female mates with all males.

To find out whether females mate monogamously or polyandrously, one needs to calculate the number of homogenetic and heterogenetic litters expected under monogamy by using equations [1] to [3], and test whether the observed number of homogenetic litters significantly less, and heterogenetic litters more, than the expected value. A one-tailed significance test is appropriate because the prediction is directional. A $\chi^2$-test is a poor choice since it is always two-tailed and its simplicity costs much of statistical power. A one-tailed test can be done by summing up the probabilities of obtaining the observed number of homogenetic and heterogenetic litters and all other frequencies representing a greater deviation from expectation. For example, if the observed number of homogenetic and heterogenetic litters is 20 and 80, respectively, and the number of homogenetic and heterogenetic litters expected under monogamy is 40 and 60, respectively, then the probability that our sampled animals are from a monogamous population is the sum of probabilities of obtaining the observed values (20 and 80) and other more extreme values such as 19 and 81, 18 and 82, 17 and 83, up to 0 and 100, from a binomial distribution of $(0.4 + 0.6)^{100}$. The sum of the probabilities in this case is smaller than 0.0001. One can therefore conclude that the result is not compatible with monogamy.

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