Genetic coupling of mate recognition systems in the genomic era.

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Abstract

The concept of Genetic Coupling in mate recognition systems arose in the 1960s as a potential mechanism to maintain coordination between signals and receivers during evolutionary divergence. At its most basic it proposed that the same genes might influence trait and preference and therefore mutations could result in coordinated changes in both traits. Since then, the concept has expanded in scope and is often used to include linkage or genetic correlation between recognition system components. Here we review evidence for genetic coupling, concentrating on proposed examples of a common genetic basis for signals and preferences. Mapping studies have identified several examples of tight genetic linkage between genomic regions influencing signals and preferences, or assortative mating. Whether this extends as far as demonstrating pleiotropy remains a more open question. Some studies, notably of *Drosophila*, have identified genes in the sex determination pathway and in pheromonal communication where single loci can influence both signals and preferences. This may be based on isoform divergence, where sex- and tissue-specific effects are facilitated by alternative spicing, or on regulatory divergence. Hence it is not clear that such examples provide compelling evidence of pleiotropy in the sense that "magic mutations" could maintain trait coordination. Rather, co-evolution may be facilitated by regulatory divergence but require different mutations or coevolution across isoforms. Reconsidering the logic of genetic coupling, it may be that pleiotropy could actually be less effective than linkage if distinct but associated variants allow molecular coevolution to occur more readily than potentially "unbalanced" mutations in single genes. Genetic manipulation or studies of mutation order effects during divergence are challenging but perhaps the only way to disentangle the role of pleiotropy versus close linkage in coordinated trait divergence.

Introduction

A critical stage in the evolution of sexual isolation is divergence in signals involved in sexual communication and their associated preferences. If a major signal trait and an associated preference both diverge between populations in a coordinated manner, assortative mating is likely to result and to contribute to reproductive isolation. Pre-mating isolation can also be favoured by divergence in seasonality, habitat choice or ecologically adapted traits that indirectly lead to assortative mating (Kopp et al. 2018), but sexual isolation in animals is often thought to emerge most directly from coordinated changes in mating signals (here interpreted broadly as any trait in one sex that can influence mate choice by the other sex) and associated preferences (Lande 1981; Panhuis et al. 2001; Ritchie 2007). Jointly, we call these coordinated signals and preferences "Mate Recognition Systems" (after Paterson 1978).

Sexual Isolation and Coevolution

Sexual isolation is often thought to be amongst the first forms of reproductive isolation to appear in many animal groups (see Shaw et al. in this volume) and therefore potentially plays an important role in animal speciation. Ultimately, mate recognition systems can diverge due to sexual selection (Ritchie 2007), including coevolutionary and antagonistic effects, or by divergent ecological selection acting directly on traits or preferences arising from environmental factors that influence signal or preference efficiency, biotic interactions between species with similar signals (reproductive interference) or reinforcing selection between hybridising species. Sexual isolation may result from mutation order effects in similar environments (Mendelson et al. 2014), and divergence could also be initiated by genetic drift (Uyeda et al. 2009).

A key factor influencing how divergence of mate recognition systems can lead to speciation is the extent to which both signal and preference evolve together within diverging populations but also jointly diverge sufficiently to generate assortative mating between populations (Ryan and Rand 1993; Rodriguez et al. 2013). Coordination between changes in signals and preferences is critical. Mutual coevolutionary processes such as Fisher's Runaway or some forms of antagonistic coevolution may maintain correlation between male and female traits in a stepwise manner. If one trait diverges first, for example through a change in environmental selection on a male signal or the spread of a mutation of large effect on the preference, then the other trait will need to "catch up" via trait modification to maintain coordination. This is potentially a slow process, especially if new mutations are required rather than standing genetic variants being available.

Genetic architecture and coevolution

The genetic architecture of signals and preferences may influence the likelihood of coevolution between them. Felsenstein's seminal work (Felsenstein 1981) highlighted the issue of recombination in speciation (see also Butlin et al. 2021). If genetically independent but interacting traits each have a simple genetic control, tight physical linkage of the loci can facilitate coevolution by maintaining association (linkage disequilibrium) between complementary allelic variants. Speciation often involves ecological adaptation, in which case signals and preferences may also need to be associated with locally adapted alleles. The necessary linkage disequilibrium (LD) can be generated in multiple ways (by mutation, drift, gene flow or selection, Charlesworth and Charlesworth (2010), chapter 8) but assortative mating is a powerful force generating associations. In whatever way it is generated, LD is broken down by recombination. Therefore, close physical linkage can play a key role in maintaining associations among alleles at different loci (Kirkpatrick 1982, Servedio and Burger 2018). Factors reducing recombination among relevant genes can be favoured in such circumstances, by

inversions or other mechanisms, facilitating the appearance of coadapted gene complexes influencing multiple traits (Ravinet et al. 2017; see chapter by Berdan et al. this volume).

”Genetic Coupling” potentially greatly facilitates coordinated changes in signals and preferences. In the 1960s Alexander proposed the concept of “genetic coupling” of traits and preferences (Alexander 1962). In this original idea, genetic coupling essentially meant genetic pleiotropy, i.e. substitution of one allele resulting in changes in multiple traits. The way the term was used specifically suggested that a new mutation could simultaneously influence both traits in a complementary manner such that substitutions of large effect would simultaneously alter the expression of both signals and preferences. This was expected to maintain some coordination and reduce the selection that would otherwise oppose new variant signals or preferences (Doherty and Hoy 1985; Ronacher 2019). Theoretically such variants could clearly influence rapid coevolution of these traits: a single mutation could alter both signal and preference, and recombination would not break the association between traits. Initial studies of interspecific hybrids, usually in acoustic communication systems, were thought to support the idea (e.g. Hoy et al. (1977)), but most were of low genetic resolution and did not provide conclusive evidence. In 1989, we (Butlin and Ritchie 1989) reviewed the evidence proposed to support the genetic coupling hypothesis and concluded that, at the time, there was no single convincing example. We also advanced several arguments to suggest that coupling was unlikely. Boake (1991) reached broadly similar conclusions. In nearly 35 intervening years, methods of genomic mapping of complex traits have been revolutionised and gene manipulation studies are becoming increasingly adept at examining (and illuminating) pleiotropy. Several studies have hinted at new evidence supporting genetic coupling and at least two have claimed positive support for genetic coupling. Here we provide an update of Butlin & Ritchie (1989). We discuss important changes in the concept of genetic coupling and the improved resolution of more recent studies.

What is “Coupling”?

Since the 1960s, the concept of “Coupling” in reproductive isolation has developed and now often refers to the interaction between multiple barriers that jointly contribute to reproductive isolation (Smadja and Butlin 2011; Butlin and Smadja 2018). If different barriers become coupled, in the sense that they operate together to reduce gene flow between a pair of populations, then the overall isolation may become more effective. Under this broad view, genetic and demographic factors, as well as natural and sexual selection, can influence the build-up of coupling. However, there is no general agreement on usage of the term: different perspectives and their implications for the role and extent of coupling are discussed in detail in Dopman et al. (this volume), as well as the possible roles of genetic linkage and pleiotropy in promoting coupling between barrier effects. “Genetic coupling”, as used in the original literature, refers to the particular case where pleiotropy leads to coordinated effects of a locus on both signals and responses, potentially leading to assortative mating. We refer to this as “Narrow-sense genetic coupling” and Table 1 places it in the context of other relevant terms and concepts (which are not mutually exclusive). “Narrow sense” Genetic Coupling concerns the evolution of a single barrier effect, because a barrier to gene flow exists only when signal and preference both diverge, and so falls outside the scope of the coupling of separate barrier effects, such as assortative mating and reduced hybrid fitness (Butlin and Smadja 2018, Dopman et al. this volume, Perspective 3). However, “Broad-sense genetic coupling”, which relies on linkage between signal and preference genes rather than pleiotropy (Table 1), may be considered part of the general coupling process under Perspectives 1 and 2 of Dopman et al. (this volume). Narrow sense genetic coupling is related and relevant to the idea of “magic traits” (Gavrilets 2004; Servedio et al. 2011) where a single gene (pleiotropy) or a single trait (“multiple effect” trait; Smadja

and Butlin 2011) influences more than one component of reproductive isolation. These mechanisms also have similarities to the case of "one allele" models of speciation (Felsenstein 1981), because they all circumvent the problem of recombination opposing speciation by breaking down allelic associations required for reproductive isolation. Finally, another relevant concept is the idea of matching traits (Kopp et al. 2018), where there are no separate signal and response traits but rather assortative mating depends on similarity between males and females for a single shared trait, for example size-assortative mating.

Concept	**Description**
Coupling I	The build-up of linkage disequilibrium among loci underlying barriers to gene exchange (Dopman et al., this volume)
Coupling II	The build-up of genome-wide linkage disequilibrium (Dopman et al., this volume)
Coupling III	The process generating a coincidence of distinct barrier effects (Dopman et al., this volume)
Broad-sense genetic coupling	A genetic association between mating signals and preferences due to tight linkage or recombination suppression (this paper).
Narrow-sense genetic coupling	A genetic association between mating signals and preferences due to pleiotropy, i.e. influence of the same allele on both traits (Alexander 1962, Butlin and Ritchie 1989, this paper)
Magic or multiple-effect trait	A single trait that influences more than one component of reproductive isolation, such as effects on both divergent adaptation and assortative mating (for example, Batesian warning colours also contributing to mate choice). The two terms are not directly equivalent (Servedio et al. 2011, Smadja and Butlin 2011, Dopman et al., this volume).
Single trait process	Divergent ecological selection on one trait indirectly leads to assortative mating (for example nest site preferences in some birds or fish) (Rice and Hostert, 1993, developed this, but called it the 'single variation model').
Single gene process	Divergence at a single locus results in one or more barriers to gene flow between populations (opposed by gene flow but not by recombination)
One-allele process	Substitution of the same allele, or evolution of a polygenic trait in the same direction, in two populations causes reproductive isolation between them, (for example the evolution of increased female choosiness) (Felsenstein 1981)
Matching trait assortment	Sexual isolation arising due to assortative mating based on phenotypes expressed in both sexes, e.g. assortative mating by body size (Kopp et al. 2018).

Table 1. Coupling and related concepts in speciation. These ideas all have in common the reduction or removal of the opposing effect of recombination on the build-up of reproductive isolation.

Arguments have been made in favour of all of these potential mechanisms. One possible case of a one-allele effect, influencing the level of choosiness, was identified in *Drosophila,* though the locus has not been characterised in detail (Ortiz-Barrientos and Noor 2005). Other possible examples have been suggested, especially the evolution of mate or habitat choice based on imprinting (Butlin et al. 2021). Servedio et al. (2011) and Smadja and Butlin (2011) argue that magic and multiple-effect traits might be common, frequently suggested potential examples including chirality in snails (though empirical studies suggest this is unlikely to be a really effective single-trait barrier, Richards et al. 2017), host choice in phytophagous insects (e.g. *Rhagoletis* fruit flies; Tait et al. (2016), *Acyrthosiphon* pea aphids; Hawthorne and Via (2001)) and wing patterns in *Heliconius* butterflies. *Heliconius* wing patterns experience divergent selection due to mimicry but are also involved in assortative mating, at least as signals (Kronforst et al. 2006; Merrill et al. 2019). Most examples are of this type, only explaining divergence in one component of the communication system, either signal or preference, being under ecological selection. The other trait also must diverge to generate assortative mating. Reproductive isolation involving matching traits might also be common (Kopp et al. 2018). Assortative mating for body size is a case in point, as exemplified by stickleback fish (Ólafsdóttir et al. 2006).

A one-allele system relies on the fixation of the same allele in different populations bringing about isolation between them. An alternative simple mechanism is where one gene substitution (of a different allele) would bring isolation due to matching effects on males and females leading to isolation Perhaps the clearest example of a potential single-gene effect in the literature is the *period* gene in *Drosophila*. This is a clock gene with extensive pleiotropic effects on most behaviours that involve intrinsic rhythmicity. (Tauber et al. 2003) completed a genetic transformation experiment, introducing the *period* gene of *D. pseudoobscura* into *D. melanogaster* and showing its effect on altered diurnal activity cycle differences could lead to assortative mating between flies with the same *period* allele and hence rhythmicity. This is a fascinating “proof of concept” study illustrating that single gene effects on pre-mating isolation (in this case allochronic isolation, a type of ‘matching trait assortment’) are possible, though the fact that the two species involved are only distantly related perhaps questions if this is a good example of a direct role in speciation. The *period* gene may also contribute to an oligogenic determination of tidal isolation between closely related marine midges (Briševac et al. 2023). If further studies confirm a key role of this gene in this timing difference, it could be a key demonstration of one gene influencing allochronic speciation in animals.

Narrow-sense genetic coupling is different from this single-gene, single-trait mechanism in that it proposes a pleiotropic effect of one gene on two traits, a signal and an associated preference. Butlin & Ritchie (1989) were unconvinced by any of the proposed examples of genetic coupling available at the time. What would constitute such an example, and is it feasible? Narrow sense genetic coupling implies that a mutation has a complementary effect on signalling and preference, that essentially the traits are controlled by the “same gene” (Shaw and Lesnick 2009). At first glance, this may seem unlikely because signals and preferences are superficially very different traits. However, in theory this is possible if there is some underlying biochemical, physiological or developmental link. For example, early work invoked the possibility of a single oscillator or common neurons underlying both the frequency of an acoustic signal and the frequency-sensitivity of a receptor (Hoy et al. 1977). An opsin gene in a fish may be under selection due to differences in colour propagation in water that influence both colour production and detection of that colour. This example is only partly hypothetical (Terai et al. 2006): opsins influence colour perception but are not known to influence colour production directly. Some colour mutants in medaka seem to influence assortative mating, although the mechanism is not clear (Fukamachi et al. 2009). The alternative to coupling would be that loci other than opsins produce matching changes in colour production that are more easily

perceived due to selected changes in opsins. The changes may still be tightly coordinated by selection but not due to a direct simultaneous effect of a single mutation on both trait and preference. Narrow-sense genetic coupling, i.e. genetic coupling as originally proposed, requires direct pleiotropic effects on signal and preference. Genetic associations due to tight linkage, co-localization in an inversion or very strong epistatic selection are not the same. However, they may be more feasible routes to rapid coevolution and have been considered as genetic coupling in much recent literature, implying only genetic linkage or genomic clustering and not necessarily pleiotropy. (Mead and Arnold 2004) used the term to include a genetic association between signal and preference generated by assortative mating, without reference to its genetic basis, but more often the term is invoked when genetic linkage between traits is tight. We will refer to this mechanism as "broad-sense genetic coupling".

The evidence.

Genetic mapping of traits, using either traditional quantitative trait locus (QTL) mapping or genomic association studies, is now much more feasible and cost-effective in a wide range of organisms. Mapping of signal traits and preferences has been completed in a number of systems, and tantalising examples of co-localization have been found. Perhaps one of the most thoroughly studied cases is QTLs for song pulse rate in Hawaiian crickets and, following an ingenious experimental design, female preferences for these songs in crosses between *Laupala kohalensis* and *L. paranigra*. Initial studies identified common QTL peaks affecting both traits (Shaw and Lesnick 2009), though these were broad and contained multiple loci. Xu and Shaw (2021) refined the mapping of the signal and preference loci and interval mapping showed that they clearly overlap, with ~3 cM difference between peaks (Figure 1). Xu and Shaw (2019) also examined different QTL on another linkage group. This chromosome also carried extremely tightly linked loci affecting song and preference, with estimates of recombination distances between them of around only 0.06 cM. These studies are an elegant demonstration of genetic linkage in the sense of a very tight, almost intimate, genetic association. It is particularly striking how tightly these pairs of major loci co-localize on two different chromosomes since both traits are polygenic, and each QTL explains ~10% of the species difference. Whether these different tight peaks include genes with pleiotropic effects (i.e. narrow-sense genetic coupling), and so the potential for a common influence on songs and preferences, remains to be established, though annotations suggest neural functions and potential pattern generator genes lie within the peaks (see also (Xu and Shaw 2020)). Pleiotropy in the case of polygenic traits is most likely where there is some common underlying functional connection, such as an oscillator.

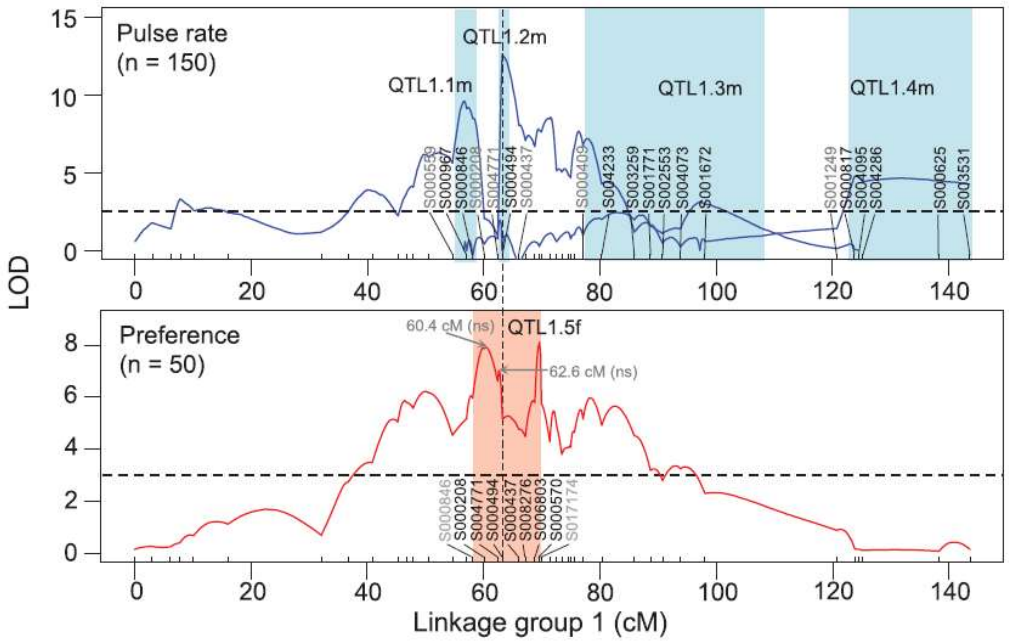

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Figure 1. QTL mapping of loci influencing song (pulse rate) and preferences for pulse rate in crosses between the Hawaiian cricket species *Laupala kohalensis* and *L. paranigra*. Shaded areas indicate ranges of mapped QTL locations and some QTLs are named (e.g. QTL1.1m; 1st on linkage group 1 in males, etc) From Xu & Shaw (2019). (Inset, *Laupala kohalensis*, photo Kerry Shaw)

225

226 Another system producing apparently compelling examples of co-localisation is provided by the
227 warning colours of *Heliconius* butterflies, which are also used as mating cues by males (they are
228 "magic" or "multiple-effect traits"). The question is whether preference genes co-localize with the
229 pattern genes. Kronforst et al. (2006) identified QTLs for colour differences between *H. cydno* and *H.*
230 *pachinus* and preferences for these patterns and found a "perfect" association between them,
231 potentially implicating pleiotropic effects of the *wingless* gene and not consistent with tight linkage,
232 unless the region also lacked recombination due to an unknown inversion. They suggested that
233 genetic coupling, while unlikely in most systems, could reflect pleiotropy here if the pigments
234 influencing wing colour are also expressed in the eyes and influence spectral sensitivity, a fascinating
235 mechanism if true. Colour pattern differences between *Heliconius cydno* and *H. melpomene* and
236 their perception have also been studied. Traits and preferences are genetically clustered (Merrill et
237 al. 2011) but detailed association mapping resolves this to a number of male choice loci, some of
238 which are tightly linked (~1cM) with the colour pattern gene *optix* (Merrill et al. 2019) (Rossi et al.
239 2020). Detailed analyses of hybrids, including expression analyses of genes associated with choice,
240 reveal that the initial larger QTL breaks down to tight linkage between loci, *optix* for colour pattern
241 and others for male mate choice (Figure 2). QTL for sex pheromones, which influence female mate
242 choice, are also clustered in the genome and may be loosely linked with some of the colour
243 patterning loci, which may mean the two signalling traits have the potential for coordinated change

(Byers et al. 2021) but do not make as compelling a case for pleiotropy as the (Kronforst et al. 2006) study.

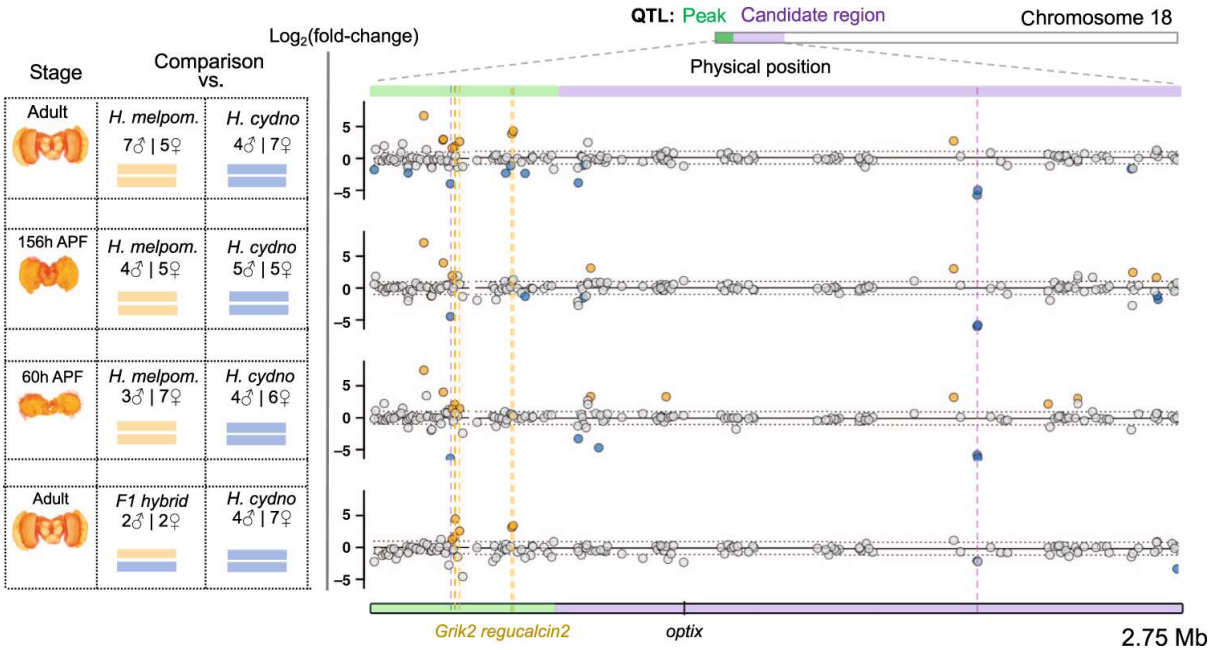

Figure 2. Differential expression analyses of brain samples from the butterflies *Heliconius melpomene* and *H. cydno* and F1 hybrids identified candidate genes involved in pattern choice (*Grik2* and *regucalcin2*) are very tightly linked to *optix*, which controls wing pattern differences. From (Rossi, Haussmann et. al. 2020) Vertical lines highlight genes with differential expression between species (see paper for further details). (Inset; *Heliconius melpomene rosina* male *x H. cydno chionus* female, photo Richard Merrill).

In some cases, assortative mating is studied directly, rather than the underlying signals and preference, which may not be known. Usually studies of assortative mating as a phenotype have led to the conclusion that this has, perhaps unsurprisingly, a polygenic basis with numerous small effect loci. This would seem to make coupling unlikely (Ting et al. 2001; Civetta and Cantor 2003). However, such loci may be more densely distributed on some chromosomes (e.g. sex chromosomes (Qvarnstrom and Bailey 2009; Abbott et al. 2017)) or co-inherited due to reduced recombination, or multiple loci might underlie a single trait that drives assortment through phenotype matching. Some studies have inferred genetic associations between traits involved, or components of assortative mating itself. An intriguing study investigated size-based assortative mating between limnetic and benthic stickleback morphs. This assortment is based on differences in morphology between the morphs, which are clearly multichromosomal and polygenic. However, (Bay et al. 2017) identified two QTLs for size-based mate choice which co-localised with some QTLs affecting body shape. Model fitting demonstrated that variation in choice QTLs explained a significant component of shape, suggesting a strong genetic correlation between the traits. Interestingly, the females' own shape was correlated with mate choice so phenotypic matching could be involved (Kopp et al. 2018). It would be interesting to see this examined in the medaka example mentioned above, another case where phenotype matching could influence assortative mating (Fukamachi, Kinoshita et al. 2009)

Using introgression experiments between *D. simulans* and *D. mauritiana*, McNiven and Moehring (2013) identified two small chromosomal regions that contained genes influencing both male and female components of assortative mating (preferences and unidentified factors influencing male species-specific attractiveness), suggesting close linkage or pleiotropy of these unidentified loci. In a study of *D. simulans* and *D. melanogaster*, deficiency mapping had identified a region including the transcription factor *fruitless* and Chowdhury et al. (2020) then used mutants to demonstrate that this locus influences female species-specific preference. This is extremely interesting: *fruitless* is studied extensively because of its dramatic effects on male courtship behaviour, including the production of courtship song (Rideout et al. 2007; Clyne and Miesenböck 2008). By alternative splicing, the *fruitless* gene produces a number of different transcripts that may be male-specific, female-specific or common to both sexes, with the potential to influence courtship song amongst other, usually sex-specific traits. Transcripts vary in exon structure and content and arise from different promoter regions and splicing variants (Parker et al. 2014). Mutations in male-specific transcripts of *fruitless* cause males to sing aberrant song or no song at all (Neville et al. 2014). The fact that *fruitless* can influence preference is tantalising, though the manner in which *fruitless* influences female behaviour is not known: it seems to involve one of the non-sex-specific transcripts produced by a different promoter region from that producing male-specific transcripts that establish male courtship behaviours. Intriguingly, this transcript does not seem to influence female receptivity to male song. This example nicely illustrates how the same gene might underlie signal and preference without the effect of any one allelic substitution acting pleiotropically. It is strictly-speaking an example of tight linkage rather than narrow-sense genetic coupling.

Another example in *Drosophila* that has been genetically dissected with great precision provides one of the most compelling cases yet for pleiotropy. Major contributors to assortative mating in the melanogaster group of *Drosophila* are cuticular hydrocarbons, and loci involved in their production and perception are well characterised. The desaturase gene family has been shown to control important changes in species-specific pheromonal components of CHCs (Jallon and Wicker-Thomas 2003; Wicker-Thomas 2007). Very elegant work has further shown how they can alter sex-specific CHC expression and change the ratio of key compounds, often produced by females and detected by males (Shirangi et al. 2009; Fang et al. 2009). Marcillac et al. (2005) took a candidate gene approach and mutated a key enzyme, *desat1*, using transposon insertion. Pheromone production was altered in both sexes, especially key sex pheromones. Males carrying the mutation were unable to distinguish the sexes, at least in the dark when pheromones are essential for sex recognition. Transposon excision affected these traits differently, perhaps suggesting the precise molecular mechanism for production and detection differed between the sexes. This was explored further by Bousquet et al. (2012) in a very elegant study. They demonstrated that *desat1* has 5 transcripts, each with the same protein but different regulatory regions. Its potential pleiotropic role was examined by generating mutants and reporters for each transcript. One promoter was strongly associated with changes in the production of saturated/desaturated CHCs and this was expressed in female oenocytes. Another was associated with male sex-discrimination, and was expressed in the antennal lobes and a sexually dimorphic glomerulus. Clearly the development of sex- and tissue-specific transcripts may well be a key to diversifying gene function in sexual communication systems and, unlike *fruitless*, this effect is primarily regulatory rather than involving both coding and regulatory variation. However, like *fruitless*, it may also be the case here that no single mutation influences signals and preferences in a coordinated fashion.

The desaturase gene family is known for its role in fatty acid production, including the cuticular hydrocarbons, and these studies suggest it also influences perception. Remarkably, a recent study provides a very similar interpretation of potential genetic coupling in Drosophila pheromones, but in

this case a gustatory receptor (Gr) locus known to influence chemical perception has been demonstrated to influence pheromone production as well. Vernier et al. (2023) measured tissue-specific expression of members of the Gr family across several tissues in Drosophila and identified that *Gr8a* is expressed in both sensory tissues and oenocytes. In males it is also expressed in the ejaculatory bulb and is suggested to be involved in the production of an inhibitory compound ("antiaphrodisiacs" are passed in the ejaculate to alter female attractiveness to rivals; Billeter and Levine (2015)). Knockdown males produce altered CHCs, though not known antiaphrodisiacs such as CVA. Knockdown females have altered mating behaviour, mating more quickly, and knockdown males are both more sexually attractive and less likely to discriminate against mated females. The authors' interpretation is that *Gr8a* is involved in regulating the behavioural responses to an inhibitory mating pheromone in females and males, and its production in males. Knockdown is a valuable method to demonstrate the role of a gene product in multiple functions but it cannot distinguish strict pleiotropy from the effects of different substitutions in the same gene.

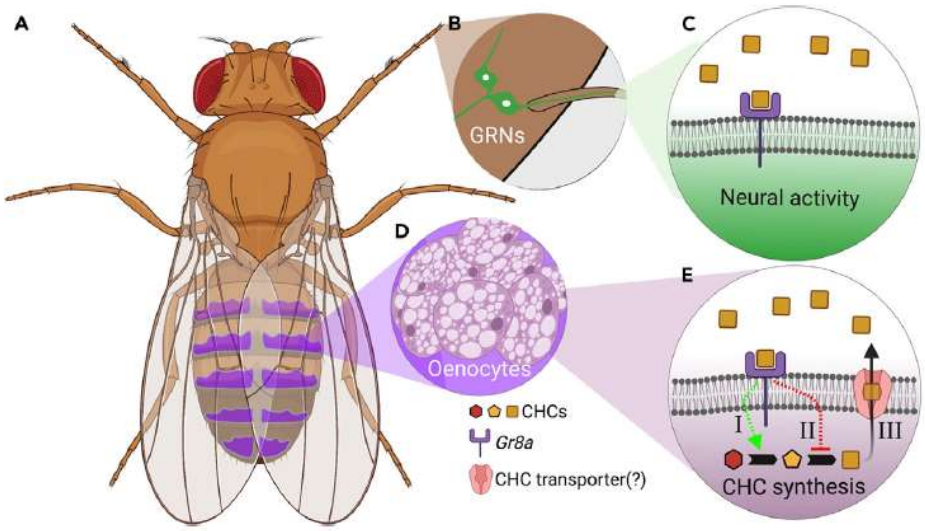

Figure 3. Model for the pleiotropic effect of *Gr8a* in both production and perception of pheromones. (A). Male fly, with oenocytes in magenta (B) *Gr8a*-expressing GRNs and in the sensory terminal tarsi (C) Gr8a functions as in inhibitory pheromone receptor in the tarsi (D) Oenocytes produce the pheromones in the abdomen (E) *Gr8a* functions as an autoreceptor in oenocytes, which regulates synthesis (I-II) and secretion (III) via feedback loops. From Vernier et al. (2023)

Other studies of pheromonal communication systems have disentangled the genetics of signal-receiver systems very clearly. Moths are ideal systems for such work and an important study system is the European corn borer, *Ostrinia nubilalis*. This system has two strains that show assortative mating due to alternate (E/Z) volatile long-range pheromone blends and has been examined for many years and in many localities. The pheromone polymorphism is mainly influenced by alleles at the *pgFAR* locus, coding for a fatty acyl reductase enzyme (Lassance et al. 2013). Pheromone discrimination was assumed to be due to candidate receptor loci expressed on the antenna, but recently very neat work (Unbehend et al. 2021) demonstrated the key locus responsible was *bric-a-brac* (*bab*), a gene including a BTB domain known to be involved in morphological pattern generation in *Drosophila*. Association analyses and CRISPR knockout demonstrated that the key difference influencing pheromone discrimination in *Ostrinia* lies in a regulatory intron rather than any exon. *pgFAR* and *bab* are on different chromosomes but a key finding from this work is that, in natural

populations, *pgFAR* and intron1 of *bab* are in strong disequilibrium and show heterozygote deficit due to assortative mating. Hence, this is a very nice example of the ability of strong assortative mating to generate linkage disequilibrium between distinct, unlinked loci affecting signal and preference. If assortative mating, perhaps combined with epistatic effects on fitness, can maintain associations even among unlinked loci, then perhaps genetic coupling is not important for the coevolution of signals and preferences. It is fascinating that *bab* has also been implicated in male UV signalling in *Colias* butterflies (Ficarrotta et al. 2022). Here the Z chromosome, in addition to carrying *bab*, also influences female preference, which is genetically associated with signalling in a hybrid zone between *C. eurytheme* and *C. philodice*, but it is not yet known whether this could be due to pleiotropy.

Whither "genetic coupling"?

This is a short and probably not exhaustive review of potential cases of genetic coupling in sexual signalling systems. It is striking that in the last few decades there have still only been two or three potential examples of genetic coupling at the level of key loci that underlie both signal and preference. So far, there is no system where the same allelic substitution has been shown to influence both traits, although that also cannot be ruled out in some cases. Therefore, narrow-sense genetic coupling, i.e. coupling due to pleiotropy rather than linkage, has not been demonstrated. Close linkage has been found, either within the same gene or in nearby genes, and this extends to cases where multiple loci influence both traits, suggesting some underlying common mechanistic connection: evidence for broad-sense genetic coupling is slowly accumulating.

Perhaps further consideration suggests that the original idea of a single mutation with coordinated effects was naïve and always unlikely. The idea of such a "magic mutation" with pleiotropic effects inducing changes in signal and preference is tantalising, but what is the likelihood that a mutation in such a gene would have a coordinated effect on both traits? Would the shift in signal necessarily match the shift in preference when the effects on some common stage in a biochemical or developmental pathway have been filtered through later steps to reach the different phenotypes? The *period* gene of *D melanogaster*, part of the clock mechanism, influences both song and female detection of song patterns, but not in a matching manner. Mutations in *per* do both influence song rhythm and disrupt preference but there is no matching of the effects of mutations such that *perL* lengthens rhythm in males and makes females prefer long rhythms (though the mutations studied to date are induced mutations rather than natural alleles) (Greenacre et al. 1993). The gene does influence a common pattern generator and so connects changes in song and recognition, but coordinated mutational effects seem unlikely. The advantage of requiring a single mutation may then be lost, because additional modifier mutations are still needed to maintain signal-response coevolution.

Fruitless and *desat1* probably indicate more convincing ways that one complex gene can influence sex- and tissue-specific functions, with multiple transcripts being able to diverge in function in different tissues involved in signal production or recognition such as oenocytes and receptors (Figure 3). Hence coordination is probably not due to a single mutation, though the alternative transcripts will be intimately linked. However, this would probably still require step-for-step changes in gene function: a mutation influencing one function has to be able to increase in frequency first and then generate selection for a corresponding change in the other function. This might limit evolution to small steps and increase the waiting time for suitable complementary mutations. In *desat1* (and *bab*) it is primarily promoter sequence divergence controlling where and how the gene is expressed (though details of how this influences coordination are not yet known). In *fruitless* there are important changes in sex-specific promoter regions, but also coding sequence divergence. (Parker et

al. 2014) showed how most exons of *fruitless* exhibit strong evidence for purifying selection, as might be expected for a key gene in the sex determination pathway with conserved gene function across Diptera, but that divergent selection was concentrated in one sex-specific exon across multiple species. Clearly identifying the mutational steps involved in adaptation and the order of their appearance during evolutionary change is a major challenge but is necessary to disentangle these questions and explore the importance of intralocus epistasis, i.e. interactions between different exons or transcripts from a single gene, or coding and regulatory divergence. Some progress is being made in identifying key mutational steps in other systems (Chan et al. 2010). CRISPR provides a fantastic opportunity to examine the role of specific mutations on multiple traits and recapitulate such steps (Karageorgi et al. 2019).

So what?

As discussed earlier and elsewhere (Dopman et al., this volume), the concept of genetic coupling has evolved and loosened. Here we have used narrow-sense genetic coupling to suggest strict pleiotropy. Broad-sense genetic coupling, we suggest, includes tight linkage, either within complex genes or between tightly-linked loci. There are increasing numbers of examples of such broad-sense genetic coupling in differences between recognition systems of sibling species, most notably *Laupala* and *Heliconius,* as well as the within-locus cases in *Drosophila*. Similarly, genomic analysis is highlighting the importance of reduced recombination, inversions and "supergenes" in ecological adaptation and speciation (Ravinet et al. 2017; Faria et al. 2019; Berdan et al. 2022). Could such linkage facilitate coordinated change just as effectively as pleiotropy? The corn borer example shows that strong assortative mating can generate linkage disequilibrium even among unlinked loci. Theory suggests that, at least in some circumstances, tight linkage is not just unnecessary for the maintenance of linkage disequilibrium but might actually impede the evolution of reproductive isolation. An intriguing recent study (Servedio and Burger 2018) modelled ecological speciation based on three loci, for an adaptation, a trait and choosiness, in the context of assessing the likelihood of "magic" versus "pseudomagic" traits (i.e. pleiotropic versus linked traits, in this context between an ecological adaptation and a male signalling trait). At least in a secondary contact scenario, recombination could facilitate the evolution of greater choosiness, though the role of the ecological adaptation is important. Whether a similar effect might occur in a non-ecological context is unclear. Similarly counterintuitive effects of recombination can occur when multiple signal traits or mutual mate choice are involved (Aubier et al. 2019; Aubier Chapter, this volume). Indeed, it is possible that pleiotropy could actually be less effective than linkage if distinct but associated loci allowed molecular coevolution to occur more quickly than an 'unbalanced' mutation in a single gene. Strict pleiotropy may prevent more favourable combinations of alleles or mutations from becoming associated by recombination, so the search for pleiotropy in mate recognition systems may ultimately be a red herring. We conclude that there is still much to be learned about the genetic bases of signals and preferences, and the co-evolutionary relationships between them, but that there is little reason to propose that strict pleiotropy is likely, or would necessarily facilitate rapid co-evolution.

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