Genetics of behavioural isolation

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Behavioural isolation is the lack of sexual attraction between individuals of two different species. This often is the first isolating barrier to evolve during speciation, and it is of utmost importance for our understanding of how species come into being. Unfortunately, there is not a single report of the cloning, isolation and characterization of any gene(s) for behavioural isolation, thus seriously impeding our understanding of how these barriers evolve. In this review, I list some of the major genetic studies which attempted the genetic dissection of behavioural isolation. I further report the general features which emerge from these studies and also why this particular phenotype, however important, is a difficult choice for normal genetic studies.

Introduction

DARWIN'S '*Origin of Species*', paradoxically, had little to say about how species come into being. It took nearly a hundred years, including the Modern Synthesis, for our understanding of speciation to congeal. Questions still persist about how speciation starts, and only in the last few decades has our understanding been on solid empirical grounding. However, our understanding of premating isolation is still incomplete. Individuals mostly prefer to mate with their own kind, which prevents collapse of species and production of hybrid swarms. But, what are the genetic mechanisms by which this phenomenon is brought about? How many genes are usually involved? What is the nature of these genes? What role does positive natural selection play in this phenotype? What, if any, are the predominant phenotypes producing this behaviour? All of these questions remain largely unanswered. Premating isolation obviously involves sexual selection, a subject that I will not elaborate upon because several excellent reviews exist[1,2]. Instead, I will concentrate on behavioural isolation.

What is behavioural isolation?

Individual animals of a particular species preferably mate with individuals of the same species. This phenomenon of

preference for conspecific over heterospecific mates is called behavioural isolation or ethological isolation. Behavioural isolation therefore is a part of the broader prezygotic isolating barriers acting on any particular species, similar to ecological and mechanical isolating barriers[3]. Behavioural isolation also differs from sexual isolation in a subtle but significant way. Sexual isolation can act after copulation (gamete transfer), but before fertilization (gamete fusion) can happen. There are many such mechanisms in animals which prevent fusion of gametes even after mating, like conspecific sperm precedence[4] and these mechanisms are termed post-mating prezygotic isolating barriers[3]. In other words, behavioural isolation is a measure of all the mechanisms which prevent heterospecific attraction resulting in failure of successful courtship and/or copulation between individuals of two different species.

Why look for genes responsible for behavioural isolation?

There are important reasons for the search of genes responsible for behavioural isolation. Foremost is their role in the formation of new species. How do new species form, is a fundamental question in biology since it is the only process by which life diversified on earth. Speciation, which Darwin called the 'mystery of mysteries' is still a little-understood process. In the past few decades our knowledge of post-zygotic isolation has vastly improved[5]. But post-zygotic isolation, generally, is a mechanism by which previously-formed species maintain their species identities by preventing the formation of viable hybrids, and these genetic mechanisms do not throw light on the processes that lead to the formation of new species. Behavioural isolation generally evolves before post-zygotic isolation but can also be strengthened by selection against maladaptive hybrids (a process called reinforcement). Therefore, it can be a component of isolating mechanisms which initiate as well as complete reproductive isolation between two species. Moreover, behavioural isolation seems to be one of the first barriers to gene flow and therefore working out its genetic basis can shed light on how species come into being. The evolution of behavioural isolation in sister taxa also brings about the evolution of a new behaviour, a somewhat under-appreciated fact in the literature. We do not have a proper understanding of the genetic changes needed for a

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new behaviour to evolve. Moreover, behavioural isolation often involves the divergence of female mate preference in closely related species. This produces variation in sexual behaviour (sometimes in both the sexes) among these species. There is a strong evidence that behavioural isolation is often strengthened in sympatric taxa compared to allopatric ones[6], indicating that reinforcement has had a role to play. In addition to all of these barriers to gene flow, our understanding of how sexual selection influences behavioural isolation[7] is also incomplete. All of these mechanisms are poorly understood at the genetic level because no gene(s) have been cloned for either female mate preference and/or reinforcement. Therefore, the hunt for gene(s) for behavioural isolation is of paramount importance.

How to detect behavioural isolation?

Detecting the presence of behavioural isolation is relatively easy. If closely related species are sympatric, and yet do not produce hybrids, one can reliably infer the presence of behavioural isolation. This can be measured in a laboratory with relatively simple assays (such as no-choice, multiple choice, etc.) and measuring different parameters like latency of courtship[8], time taken to accept mates[9] and/or checking whether sperm transfer has happened or not. However, to understand the mechanism of behavioural isolation and to do proper genetic dissection, one has to figure out the traits involved in this isolation and which, unfortunately, many studies do not. This is understandable, since it is much easier to determine the presence of behavioural isolation than to establish the traits involved because of the variation of traits involved in effecting this isolation. Variations can range from courtship song, pheromones and mating calls to subtle morphological changes. In the absence of such traits, indirect genetic methods have been widely used which score for the acceptance of mates with various backcrossed genotypes which are then transformed into classical quantitative trait loci (QTLs) studies which indicate the chromosomal regions responsible for behavioural isolation also giving an estimate of the number of genes involved. One of the widely used methods in these studies is the use of introgression analysis. This process involves repeated backcrossing of hybrid progeny to any one of their parental types so that relatively small and defined chromosomal fragments can be introgressed into the genetic background of a different species and/or strains. The phenotypes of these introgressed lines are then determined for behavioural isolation. The introgressed chromosomal fragments are then tracked by suitable molecular markers and based on the phenotypes shown, are then scored to yield a QTL map indicating their chromosomal locations. If substantial genomic information is available for the taxa under study and the identified chromosomal regions are small enough, then these QTL maps can also indicate a list of candidate genes for behavioural isolation. Below is an overview of some of the different genetic studies undertaken in various groups of animals for behavioural isolation.

Studies in *Drosophila*

Drosophila remain the best-studied model organisms with the widest availability of genetic tools, hence the bulk of the genetic studies for behavioural isolation, unsurprisingly, has been done in this group[10,11]. The suitability of *Drosophila* to mutagenesis studies has enabled biologists to uncover a host of genes which influence various aspects of both male and female mating behaviour (for an exhaustive list see ref. 10). However, genes responsible for a current phenotype does not always mean that they were also responsible for the evolution of the same phenotype. Therefore, we still lack a genetic study identifying gene(s) responsible for behavioural isolation even in *Drosophila*. However, these genes can certainly illuminate the phenotypes involved and the nature of the genetic basis for evolution of behavioural isolation.

One of the most well-characterized studies of genetics of intraspecific behavioural isolation in *D. melanogaster* involves the M and Z strains[12]. The M form represents *D. melanogaster* from its cosmopolitan distribution but the Z form comes only from Zimbabwe. Females of the Z form discriminate against males from the M form. This is in spite of showing no post-zygotic isolation between the two, an indication that reinforcement probably did not play an important role in the evolution of this phenotype[13]. This discriminating behaviour of the Z females have been mapped to four different regions in the two arms of the third chromosome[14].

Similar studies on the variation of mate preference within various populations of *D. melanogaster* have also yielded potential loci for variation in female cuticular hydrocarbons (CHC). CHC have been implicated to play a major role in mate preference and thereby in behavioural isolation (for a review see ref. 15). In Afro-Caribbean *D. melanogaster* females, the major CHC are 5,9-heptacosadiene (5,9-HD), whereas, almost everywhere else, in the so-called 'cosmopolitan' population, it is 7,11-HD[16]. The loci controlling this variation were identified by Ferveur et al.[16] to be on chromosome 3. Coyne et al.[17] further refined the interval and later work confirmed it to be a *desaturase*-2 gene (a $\Delta 9$ fatty acid desaturase)[18,19]. However, whether this genetic variation is the cause of the behavioural isolation between the two populations of *D. melanogaster* remains controversial[15,20].

The use of *D. melanogaster* as a model species to study the genetic basis of interspecific behavioural isolation has an insurmountable barrier and that is its inability to produce viable hybrids with most of its sibling species[21].

Therefore, most of these types of studies involve other species of *Drosophila* than *D. melanogaster*. Of particular interest has been the 'Island endemic' species like *D. mauritiana*, *D. sechellia* and *D. santomea* which are found in the islands of Mauritius[22], Seychelles[23] and São Tomé[24] respectively. *Drosophila simulans* along with *D. mauritiana* and *D. sechellia* form the simulans clade which diverged around quarter of a million years ago[25]. Although relatively young, the species in this clade have diverged enough to show post-zygotic isolation. Hybridization between these species produces sterile males (according to Haldane's rule) but, conveniently for genetic analysis, produces fertile females. Coyne[26] first identified that the phenotype causing behavioural isolation between *D. simulans* and *D. mauritiana* was the species-specific pheromonal difference. However, Coyne and Charlesworth[27] found that the cause of behavioural isolation between *D. mauritiana* females and *D. sechellia* males had an additional component, probably female behaviour. By screening a large number of introgression lines McNabney[28] found a QTL of major effect on the X chromosome for this isolating mechanism.

D. simulans and *D. sechellia* show asymmetric behavioural isolation where *D. sechellia* females are more discriminating against heterospecific mating than *D. simulans* females. By backcrossing F1 hybrids to *D. sechellia* males, Coyne[29] found QTLs in the second and third chromosome for this particular behaviour.

D. santomea, another island endemic, has also been used for several genetic studies of behavioural isolation. This species diverged from *D. yakuba* around 400,000 years ago[30]. There is behavioural isolation acting upon the *D. Santomea* males too, as they do not court *D. yakuba* females vigorously. Moehring *et al.*[31] found that female *D. santomea* rejection of heteospecific males mapped to two regions on the X chromosome and one on the third.

One of the more successful genetic studies for the isolation of genetic loci responsible for behavioural isolation by female mate preference, has been done in the species pair *D. ananassae* and *D. pallidosa*[32]. Females of both species exhibit strong discrimination against heterospecific males, but hybrids show a preference for *D. ananassae* males. By creating introgression lines of very small heterospecific chromosomal regions in the other species' background and then by screening against *D. ananassae* males, Doi *et al.*[32] found a relatively small region responsible for the female preference of *D. ananassae*. This locus mapped very close to where the *Delta* locus and a neurogenic gene are in *D. melanogaster*[33].

Studies in Lepidoptera

Lepidoptera, especially various sibling species of moths, have been a rich source of studies for behavioural isola-

tion[34]. The European corn borer, *Ostrinia nubilalis*, is a pyralid moth and a pest of maize which is abundant in North America having been introduced from Europe at the turn of the last century. The behavioural isolation between two sympatric races of this moth is caused by differences in the ratio of two pheromones produced by the females[35]. By using gas chromatography of pheromone glands and single sensillum recordings of reciprocal crosses, it was found that the behaviour is controlled by three different genetic regions. The pheromone production is controlled by an autosomal factor, the pheromone-responding olfactory cells are controlled by another autosomal factor and the behavioural response towards these pheromonal differences is controlled by a sex-linked locus[35].

Spodoptera latifascia and *S. descoinsi* are two closely related noctuid moth species which occur in sympatry in parts of French Guiana. The major component of behavioural isolation between them is also effected by pheromonal blends[36]. This is controlled by a pair of major effect alleles from a single locus[36]. Similarly, in another noctuid moth species pair of *Heliothis subflexa* and *H. virescens*, the pheromonal blend of three chemicals plays a major role in eliciting behavioural isolation[37]. Gould *et al.*[38] found that the genetic basis of this variation maps to a single QTL which codes for at least four tightly-linked odorant receptor genes.

Studies in crickets

Laupala kohalensis and *L. paranigra* are two of several species of crickets endemic to the Hawaiian Islands. Although morphologically very similar, they are distinct in the male mating songs which are acoustically distinct especially in the pulse rates. This variation was found to have a genetic basis and the differences mapped to at least eight different loci[39].

Studies in wasps

The parasitoid wasp genus *Nasonia* consists of four closely related species[40,41] and exhibits female discriminatory behaviour against heterospecific males[40,42,43] predominantly through pheromones[43], although there are differences in male courtship behaviour as well[44]. In a recent study[45], the genetic basis of the pheromone evolution in *N. vitripennis*[43] was pinned down to three closely linked genes. This is the first demonstration of the genetic basis of evolution of pheromones in closely related species and the mechanism was confirmed by RNAi knockdown studies. Although this study did not look for genes responsible for behavioural isolation, *per se*, it nevertheless gives us the first reference of how pheromones evolve which obviously play an important role in the evolution of behavioural isolation.

What do these genetic studies tell us about behavioural isolation?

The first important insight that many of these studies give is where the genes for behavioural isolation are located. Most of the genetic regions so far isolated, at least in fruitfiles, are near regions of low recombination. Genetic loci determining *D. santomea* female's rejection of *D. yakuba* males are near the centromere[46], while that between *D. simulans* and *D. mauritiana*[47] and between *M* and *Z* forms of *D. melanogaster* are near the telomeres[14]. Behavioural isolation studies between the species pairs *D. ananassae/D. pallidosa*[48] and *D. pseudoobscura/D. persimilis*[49] indicate that the chromosomal regions responsible are near inversion polymorphisms which are also regions of low recombination. Whether this pattern is true for most species or is an artefact of looking into just one taxon (*Drosophilid* flies) remains to be seen[10].

The second important feature of these studies is that they indicate the number of genes involved in these phenotypes (for a list see ref. 3). Most studies indicate that behavioural isolation is polygenic. Although there are several studies (like the corn borer and *D. pseudoobscura* and *D. persimilis*[50]) which indicate there is a single gene of large effect. However, since all of these are QTL studies, it is difficult to make a judgement call as these regions might contain more than one gene which are tightly linked (like in *Nasonia* wasps and *Heliothis* moths). The polygenic nature of these traits are intuitively easier to grasp, as such behaviours are a co-ordinated collection of many simple behaviours on part of both males and females.

The third important feature that becomes clear is that in most of these studies behavioural isolation disappears in F1 hybrids indicating that the discriminating behaviour acts recessively in hybrids. Essentially, hybrids between two behaviourally isolated species do not discriminate between any of the parental types and readily mate with each of them. This is generally true for most of the *Drosophilids* as well as *Nasonia* wasps[40]. According to Coyne and Orr[3] this probably indicates that in each of these cases the speciation event was allopatric in nature. This is because recessive alleles for female preference cannot spread if the speciation was sympatric, as there would be no evolution of assortative mating as heterozygous females would have mated with males from both species.

Conclusion

Ritchie[51] lamented over two decades ago about the various setbacks that biologists faced trying to clone loci for female mate-preference phenotypes. Unfortunately, the situation has not improved, in so far as identification of specific genes for behavioural isolation is concerned. This is not surprising since these phenotypes are complex in nature and involve multiple components across both sexes. Significantly, it involves multiple sensory systems

in animals like sight, auditory and chemosensory perceptions. Moreover, these phenotypes are inherently polygenic in nature with variations in effect sizes[52] for each genetic loci. For efficient recombination mapping and correct identification of the responsible loci these studies need to have very large sample sizes of hybrids and also need quick and efficient measuring of the phenotypes involved. Unfortunately, unlike most morphological phenotypes, none of these behavioural phenotypes are easy to score. This points to the general problem in genetics of advancing from one particular QTL to the gene(s) responsible for that particular phenotype[53]. This has often proven to be non-trivial because of the complex nature of the traits involved. However, with the advent of rapid and relatively cheap sequencing capabilities, biologists now might not be as handicapped as in the past for proper molecular markers and lack of genomic information. This makes room for cautious optimism that having enough sequence information along with proper phenotyping capabilities, biologists might have a decent candidate gene list to try functional studies and implicate genes for behavioural isolation.

1. Bakker, T. and Pomiankowski, A., The genetic basis of female mate preferences. *J. Evol. Biol.*, 1995, **8**(2), 129–171.
2. Panhuis, T. M., Butlin, R., Zuk, M. and Tregenza, T., Sexual selection and speciation. *Trends Ecol. Evol.*, 2001, **16**(7), 364–371.
3. Coyne, J. A. and Orr, H. A., *Speciation*, Sinaur Associates, Sunderland, MA: 2004, pp. 276–281.
4. Howard, D. J., Conspecific sperm and pollen precedence and speciation. *Ann. Rev. Ecol. Syst.*, 1999, **30**(1), 109–132.
5. Presgraves, D. C., The molecular evolutionary basis of species formation. *Nat. Rev. Genet.*, 2010, **11**(3), 175–180.
6. Coyne, J. A. and Orr, H. A., Patterns of speciation in *Drosophila* revisited. *Evolution*, 1997, **51**(1), 295–303.
7. Ritchie, M. G., Sexual selection and speciation. *Ann. Rev. Ecol. Evol. Syst.*, 2007, **38**, 79–102.
8. Anholt, R. R. and Mackay, T. F., Quantitative genetic analyses of complex behaviours in *Drosophila*. *Nature Rev. Genet.*, 2004, **5**(11), 838–849.
9. Velthuis, B. J., Yang, W. C., van Opijnen, T. and Werren, J. H., Genetics of female mate discrimination of heterospecific males in Nasonia (Hymenoptera, Pteromalidae). *Anim. Behav.*, 2005, **69**, 1107–1120.
10. Laturney, M. and Moehring, A. J., The genetic basis of female mate preference and species isolation in Drosophila. *Int. J. Evol. Biol.*, 2012, 2012.
11. Nanda, P. and Singh, B. N., Behavioural reproductive isolation and speciation in *Drosophila*. *J. Biosci.*, 2012, **37**(2), 359–374.
12. Wu, C. I., Hollocher, H., Begun, D. J., Aquadro, C. F., Xu, Y. J. and Wu, M. L., Sexual isolation in *Drosophila melanogaster* – a possible case of incipient speciation. *Proc. Natl. Acad. Sci. USA*, 1995, **92**(7), 2519–2523.
13. Hollocher, H., Ting, C.-T., Wu, M.-L. and Wu, C.-I., Incipient speciation by sexual isolation in *Drosophila melanogaster*: extensive genetic divergence without reinforcement. *Genetics*, 1997, **147**(3), 1191–1201.
14. Ting, C.-T., Takahashi, A. and Wu, C.-I., Incipient speciation by sexual isolation in Drosophila: concurrent evolution at multiple loci. *Proc. Natl. Acad. Sci.*, 2001, **98**(12), 6709–6713.
15. Smadja, C. and Butlin, R. K., On the scent of speciation: the chemosensory system and its role in premating isolation. *Heredity*, 2008, **102**(1), 77–97.

16. Ferveur, J.-F., Cobb, M., Boukella, H. and Jallon, J.-M., World-wide variation in *Drosophila melanogaster* sex pheromone: behavioural effects, genetic bases and potential evolutionary consequences. *Genetica*, 1996, **97**(1), 73–80.

17. Coyne, J. A., Wicker-Thomas, C. and Jallon, J., A gene responsible for a cuticular hydrocarbon polymorphism in *Drosophila melanogaster*. *Genet. Res.*, 1999, **73**(03), 189–203.

18. Dallerac, R., Labeur, C., Jallon, J.-M., Knipple, D. C., Roelofs, W. L. and Wicker-Thomas, C., A Δ9 *desaturase* gene with a different substrate specificity is responsible for the cuticular diene hydrocarbon polymorphism in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA*, 2000, **97**(17), 9449–9454.

19. Takahashi, A., Tsaur, S.-C., Coyne, J. A. and Wu, C.-I., The nucleotide changes governing cuticular hydrocarbon variation and their evolution in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA*, 2001, **98**(7), 3920–3925.

20. Coyne, J. A. and Elwyn, S., Does the desaturase-2 locus in *Drosophila melanogaster* cause adaptive and sexual isolation? *Evolution*, 2006, **60**(2), 279–291.

21. Moulin, B., Aubin, T. and Jallon, J.-M., Why there is a one-way crossability between *D. melanogaster* and *D. simulans*? *Genetica*, 2004, **120**(1–3), 285–292.

22. Tsacas, L. and David, J. R., *Drosophila mauritianan*. sp. dugroupe melanogaster de l'Ile Maurice. Bulletin de la Societe Entomologique de France, 1974, **79**, 42–46.

23. Tsacas, L. and Baechli, G., *Drosophila sechellia*, n.sp., huitiemeespece du sous-goupe melanogaster des Iles Sechelles (Diptera, Drosophilidae). *Revue francaised'entomologie. Nouvelle Serie*, 1981, **3**(4), 146–150.

24. Lachaise, D., Evolutionary novelties in islands: *Drosophila santomea*, a new melanogaster sister species from São Tomé. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.*, 2000, **267**(1452), 1487–1495.

25. Kliman, R. M. *et al.*, The population genetics of the origin and divergence of the *Drosophila simulans* complex species. *Genetics*, 2000, **156**(4), 1913–1931.

26. Coyne, J. A., Genetics of sexual isolation between two sibling species, *Drosophila simulans* and *Drosophila mauritiana*. *Proc. Natl. Acad. Sci. USA*, 1989, **86**(14), 5464–5468.

27. Coyne, J. A. and Charlesworth, B., Genetics of a pheromonal difference affecting sexual isolation between *Drosophila mauritiana* and *D. sechellia*. *Genetics*, 1997, **145**(4), 1015–1030.

28. McNabney, D. R., The genetic basis of behavioral isolation between *Drosophila mauritiana* and *D. sechellia*. *Evolution*, 2012, **66**(7), 2182–2190.

29. Coyne, J. A., Genetics of sexual isolation in females of the *Drosophila simulans* species complex. *Genet. Res.*, 1992, **60**(1), 25–31.

30. Llopart, A., Elwyn, S., Lachaise, D. and Coyne, J. A., Genetics of a difference in pigmentation between *Drosophila yakuba* and *Drosophila santomea*. *Evolution*, 2002, **56**(11), 2262–2277.

31. Moehring, A. J., Llopart, A., Elwyn, S., Coyne, J. A. and Mackay, T. F. C., The genetic basis of prezygotic reproductive isolation between *Drosophila santomea* and *D. yakuba* due to mating preference. *Genetics*, 2006, **173**(1), 215–223.

32. Doi, M., Matsuda, M., Tomaru, M., Matsubayashi, H. and Oguma, Y., A locus for female discrimination behavior causing sexual isolation in *Drosophila*. *Proc. Natl. Acad. Sci. USA*, 2001, **98**(12), 6714–6719.

33. Vässin, H. and Campos-Ortega, J. A., Genetic analysis of delta, a neurogenic gene of *Drosophila melanogaster*. *Genetics*, 1987, **116**(3), 433–445.

34. Lassance, J.-M., Journey in the *Ostrinia* world: from pest to model in chemical ecology. *J. Chem. Ecol.*, 2010, **36**(10), 1155–1169.

35. Roelofs, W. *et al.*, Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. *Proc. Natl. Acad. Sci.*, 1987, **84**(21), 7585–7589.

36. Monti, L., Génermont, J., Malosse, C. and Lalanne-Cassou, B., A genetic analysis of some components of reproductive isolation between two closely related species, *Spodoptera latifascia* (Walker) and *S. descoinsi* (Lalanne-Cassou and Silvain) (Lepidoptera: Noctuidae). *J. Evol. Biol.*, 1997, **10**(1), 121–134.

37. Groot, A. T., Horovitz, J. L., Hamilton, J., Santangelo, R. G., Schal, C. and Gould, F., Experimental evidence for interspecific directional selection on moth pheromone communication. *Proc. Natl. Acad. Sci. USA*, 2006, **103**(15), 5858–5863.

38. Gould, F. *et al.*, Sexual isolation of male moths explained by a single pheromone response QTL containing four receptor genes. *Proc. Natl. Acad. Sci.*, 2010, **107**(19), 8660–8665.

39. Shaw, K. L., Polygenic inheritance of a behavioral phenotype: interspecific genetics of song in the Hawaiian cricket genus Laupala. *Evolution*, 1996, **50**(1), 256–266.

40. Raychoudhury, R. *et al.*, Behavioral and genetic characteristics of a new species of *Nasonia*. *Heredity*, 2010, **104**(3), 278–288.

41. Raychoudhury, R., Baldo, L., Oliveira, D. C. S. G. and Werren, J. H., Modes of acquisition of *Wolbachia*: horizontal transfer, hybrid introgression and co-divergence in the *Nasonia* species complex. *Evolution*, 2009, **63**(1), 165–183.

42. Buellesbach, J., Greim, C., Raychoudhury, R. and Schmitt, T., Asymmetric assortative mating behaviour reflects incomplete prezygotic isolation in the *Nasonia* species complex. *Ethology*, 2014, **120**(8), 834–843.

43. Buellesbach, J., Gadau, J., Beukeboom, L., Echinger, F., Raychoudhury, R., Werren, J. and Schmitt, T., Cuticular hydrocarbon divergence in the jewel wasp *Nasonia*: evolutionary shifts in chemical communication channels? *J. Evol. Biol.*, 2013, **26**(11), 2467–2478.

44. Assem, J. and Werren, J. H., A comparison of the courtship and mating behavior of three species of *Nasonia* (Hymenoptera: Pteromalidae). *J. Insect Behav.*, 1994, **7**(1), 53–66.

45. Niehuis, O. *et al.*, Behavioural and genetic analyses of *Nasonia* shed light on the evolution of sex pheromones. *Nature*, 2013, **494**(7437), 345–348.

46. Moehring, A. J., Llopart, A., Elwyn, S., Coyne, J. A. and Mackay, T. F. C., The genetic basis of prezygotic reproductive isolation between *Drosophila santomea* and *D. yakuba* due to mating preference. *Genetics*, 2006, **173**, 215–223.

47. Moehring, A. J., Li, J. A., Schug, M. D., Smith, S. G., deAngelis, M., Mackay, T. F. C. and Coyne, J. A., Quantitative trait loci for sexual isolation between *Drosophila simulans* and *D. mauritiana*. *Genetics*, 2004, **167**(3), 1265–1274.

48. Sawamura, K., Zhi, H., Setoguchi, K., Yamada, H., Miyo, T., Matsuda, M. and Oguma, Y., Genetic analysis of female mating recognition between *Drosophila ananassae* and *Drosophila pallidosa*: application of interspecific mosaic genome lines. *Genetica*, 2008, **133**(2), 179–185.

49. Noor, M. A., Grams, K. L., Bertucci, L. A. and Reiland, J., Chromosomal inversions and the reproductive isolation of species. *Proc. Natl. Acad. Sci. USA*, 2001, **98**(21), 12084–12088.

50. Ortíz-Barrientos, D. and Noor, M. A., Evidence for a one-allele assortative mating locus. *Science*, **2005**, **310**(5753), 1467–1467.

51. Ritchie, M. G., Setbacks in the search for mate-preference genes. *Trends Ecol. Evol.*, 1992, **7**(10), 328–329.

52. Moehring, A. J. and Mackay, T. F., The quantitative genetic basis of male mating behavior in *Drosophila melanogaster*. *Genetics*, 2004, **167**(3), 1249–1263.

53. Drinkwater, N. R. and Gould, M. N., The long path from QTL to gene. *PLoS Genet.*, 2012, **8**(9), p. e1002975.