

- 78 Harrison, R.G. (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends Ecol. Evol.* 4, 6–12
- 79 Noor, M.A.F. (1999) Reinforcement and other consequences of sympatry. *Heredity* 83, 503–508
- 80 Day, T. (2000) Sexual selection and the evolution of costly female preferences: spatial effects. *Evolution* 54, 715–730
- 81 Cain, M.L. *et al.* (1999) Reinforcing selection is effective under a relatively broad set of conditions in a mosaic hybrid zone. *Evolution* 53, 1343–1353
- 82 Sanderson, N. (1989) Can gene flow prevent reinforcement? *Evolution* 43, 1123–1235
- 83 Kirkpatrick, M. and Servedio, M.R. (1999) The reinforcement of mating preferences on an island. *Genetics* 151, 865–884
- 84 Servedio, M.R. and Kirkpatrick, M. (1997) The effects of gene flow on reinforcement. *Evolution* 51, 1764–1772
- 85 Kelly, J.K. and Noor, M.A.F. (1996) Speciation by reinforcement: a model derived from studies of *Drosophila*. *Genetics* 143, 1485–1497
- 86 Stam, P. (1982) The evolution of reproductive isolation in closely adjacent plant populations through differential flowering time. *Heredity* 50, 105–118
- 87 Drossel, B. and McKane, A. (2000) Competitive speciation in quantitative genetic models. *J. Theor. Biol.* 204, 467–478
- 88 Higashi, M. *et al.* (1999) Sympatric speciation by sexual selection. *Nature* 402, 523–526
- 89 Bush, G.L. (1994) Sympatric speciation in animals: new wine in old bottles. *Trends Ecol. Evol.* 9, 285–288
- 90 Turner, G.F. and Burrows, M.T. (1995) A model of sympatric speciation by sexual selection. *Proc. R. Soc. London B Biol. Sci.* 260, 287–292
- 91 Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*, Clarendon Press
- 92 van Doorn, G.S. (1998) Sympatric speciation and extinction driven by environment dependent sexual selection. *Proc. R. Soc. London B Biol. Sci.* 265, 1915–1919
- 93 Abrams, P.A. (1980) Consumer functional response and competition in consumer-resource systems. *Theor. Popul. Biol.* 17, 80–102
- 94 Spencer, H.G. *et al.* (1986) A theoretical investigation of speciation by reinforcement. *Am. Nat.* 128, 241–262
- 95 Case, T.J. and Taper, M.L. (2000) Interspecific competition, environmental gradients, gene flow, and the coevolution of species borders. *Am. Nat.* 155, 583–605
- 96 Maynard Smith, J. (1966) Sympatric speciation. *Am. Nat.* 100, 637–650
- 97 Felsenstein, J. (1981) Skepticism towards Santa Rosalia, or why are there so few kinds of animals. *Evolution* 35, 124–138

The genetics of species differences

H. Allen Orr

Species are separated by reproductive isolation as well as by more 'ordinary' differences in morphology and behavior that play no necessary role in blocking gene flow. Although a great deal is now known about the genetics of reproductive isolation, we are only beginning to understand the genetic basis of ordinary phenotypic differences between species. I review what is known about the number of genes involved in such differences, as well as about the role of major genes and epistasis in the evolution of these differences. I also compare and contrast these findings with those on the genetics of reproductive isolation.

If we compare individuals from the same species with those from different species, we see two kinds of differences. First, members of separate species are reproductively isolated and, second, they often differ dramatically in morphology and behavior. According to the BIOLOGICAL SPECIES CONCEPT (see Glossary), these differences are themselves different. The first is a necessary part of what it means to be species whereas the second is not. Despite this, it is clear that REPRODUCTIVE ISOLATION and 'ordinary' phenotypic differences tend to go hand-in-hand, and there is good reason to think that this association is partly causal. In fact, there is good reason to think the causes run in both directions: phenotypic evolution might often give rise to reproductive isolation¹, whereas reproductive isolation might often preserve phenotypic differences when taxa come into contact with each other².

Although the genetics of reproductive isolation has been studied intensively, that of ordinary species differences (i.e. differences in traits expressed within pure species and that play no necessary role in blocking gene flow) has not. The reason does not seem primarily technical as the two kinds of study largely involve the same experimental approaches. Instead, the reason probably reflects the above difference in

logical status: if species are things that are reproductively isolated, the genetic study of SPECIATION will sensibly start with such isolation. However, genetic studies of ordinary species differences have now progressed far enough to allow at least some preliminary conclusions.

The first survey of the genetics of species differences appeared in 1938, with J.B.S. Haldane's 'The nature of interspecific differences'³. Although the problem he discussed was largely dropped (little of much relevance appeared over the next 45 years; but see Ref. 4) two important developments occurred over the past 15 years. The first was the rise of QUANTITATIVE TRAIT LOCUS (QTL) and related association-mapping strategies. We now possess the polymorphic neutral molecular markers and the body of statistical theory required to map the genes underlying arbitrary trait differences in arbitrary species, at least roughly⁵. The second was the appearance of molecular tools (e.g. germline transformation) that allow us, in favorable cases, to confirm the identity of a particular gene underlying a phenotypic difference and, in especially favorable cases, to identify the actual nucleotide changes ('Quantitative Trait Nucleotides' or QTNs) involved.

Despite these technical advances, current questions about species differences largely remain the same as in Haldane's day. They are questions about genetic architecture: How many genes are involved? How large are their phenotypic effects? Where are these genes and what are their functions in development? And what are the roles, if any, of dominance and epistasis? This emphasis on numbers of genes and sizes of effects reflects one of the oldest problems in evolutionary biology – the complexity of

H. Allen Orr
Dept of Biology,
University of Rochester,
Rochester, NY 14627,
USA.
e-mail:
aorr@mail.rochester.edu

Table 1. Recent genetic analyses of species differences^{a,b}

Species ^c	Character	Number of genes	Refs	
<i>Drosophila simulans</i> – <i>D. sechellia</i>	Adult toxin resistance	>5	9	
	Larval toxin resistance	>3	47	
	Oviposition site preference	>2	48	
	Fine larval hairs	1	8	
	Posterior lobe area	>11	17	
	Posterior lobe shape	>9	17	
	Sex comb tooth number	>4	17	
	Testis length	>7	17	
	Cyst length	>3	17	
	Tibia length	>5	17	
	Male pheromone	>5	49	
	<i>D. simulans</i> – <i>D. mauritiana</i>	Posterior lobe size/shape	>8	11
		Posterior lobe size/shape	>19	10
Anal plate bristle number		>6	34	
Clasper bristle number		>1	34	
Sex comb tooth number		>2	34	
Fifth sternite		>1	34	
Anal plate area		>3	34	
Posterior lobe area		>8	34	
<i>D. mauritiana</i> – <i>D. sechellia</i>		Female pheromones	>6	50
		<i>D. melanogaster</i> –African/ Caribbean Cosmopolitan	Cuticular hydrocarbon profile	1
<i>D. simulans</i> – <i>D. melanogaster</i>	Female pheromones		>5	51
<i>D. auraria</i> – <i>D. biauaria</i>	Male courtship song	>2	52	
<i>Nasonia giraulti</i> – <i>N. vitripennis</i>	Wing size	>2	31	
<i>Laupala paranigra</i> – <i>L. kohalensis</i>	Song pulse rate	>8 ^b	53	
	<i>Microseris douglasii</i> – <i>M. bigelovii</i>	Pappus part number	>1	54
<i>Mimulus lewisii</i> – <i>M. cardinalis</i>		Anthocyanin concentration	>1	13
	Carotenoid concentration	>3	13	
	Lateral petal width	>8	13	
	Corolla width	>8	13	
	Corolla projected area	>7	13	
	Upper petal reflexing	>5	13	
	Lateral petal reflexing	>4	13	
	Nectar volume	>3	13	
	Stamen length	>7	13	
	Pistil length	>7	13	
	Corolla aperture width	>8	13	
	Corolla aperture height	>4	13	
	<i>M. guttatus</i> – <i>M. platycalyx</i>	Flower length	>1	32
Pistil length		>1	32	
Long stamen length		>3	32	
Short stamen length		>3	32	
Anther–stigma separation		>2	32	
<i>M. guttatus</i> – <i>M. micranthus</i>	Bud growth rate	>8 ^b	55	
<i>Mimulus</i> spp. ^d	In (flowering time)	>1 ^b	33	
	Corolla width	>9 ^b	33	
	Corolla length	>10 ^b	33	
	Stamen length	>8 ^b	33	
	Pistil length	>13 ^b	33	
	Stigma–anther separation	>5 ^b	33	
<i>Lactuca canadensis</i> – <i>L. graminifolia</i> ^e	Pollen color	1	14	
	Leaf lobing	1	14	
	Growth habit	1	14	

the genetic changes underlying phenotypic evolution⁶. Although there is no doubt that early champions of simple macromutational change were too extreme, a good case can be made for the founders of the modern synthesis being similarly extreme in their advocacy of genetically complex micromutational change. And a very good case can also be made that the founders arrived at their micromutational consensus on the basis of thin evidence indeed⁶. QTL analysis has promised to change all this, opening the door to the routine characterization of the genetics of species differences.

Here, I review what we have learned about the basis of species differences. I also contrast what we now know about the genetic bases of ordinary differences with what we know about the genetic bases of reproductive isolation. I restrict attention to recent studies as a review of the older literature can be found in Ref. 6. As we will see, our answers to several key questions remain frustratingly uncertain. I will argue that this partly reflects several conceptual confusions that plague the literature on species differences.

Genetic architecture of species differences

Is the genetic basis of species differences simple or complex? Here, we immediately encounter the first of our confusions and one that, although obvious, has given rise to much misunderstanding. The genetics of species differences can be 'simple' or 'complex' in at least three different ways: (1) the number of genetic changes underlying a phenotypic difference can be small or big; (2) genes of large effect can be present or absent; and (3) complicated epistatic interactions can be present or absent. Unfortunately, these distinctions have little in common. A species difference that involves many genes might, for instance, involve a major gene. Less intuitively, a species difference that involves a single gene might not involve a major gene.

Our present task is to consider the data that bear on each of these distinctions. As we will see, each suffers its own species of confusion.

Number of factors underlying species differences

Table 1 lists recent genetic studies of species differences, including analysis of 54 traits, distributed over 22 studies. Nearly half of the traits studied are in animals (mostly *Drosophila* spp.) and half are in plants (mostly *Mimulus* spp.). The overwhelming majority of studies involve mapping experiments, although a few are BIOMETRIC. Although response to artificial selection within species often involves many genes⁷, the most striking feature of the between-species data shown in Table 1 is its lack of consistency: there is a mix of results, ranging from one to many genes. This variation is best appreciated by focusing on three particularly thorough analyses, all in *Drosophila*. In the first, Sucena and Stern⁸

^aTo be included, studies must have been published after Ref. 6 and must involve 'ordinary' phenotypic differences between taxa (i.e. hybrid sterility and inviability are excluded). Crosses involving domesticated species were not included.

^bBiometric estimates: reflect point estimates, not lower bounds on 95% confidence intervals.

^cCommon names: *Drosophila* spp., Fruit flies; *Lactuca* spp., Lettuce; *Laupala* spp., Hawaiian cricket; *Mimulus* spp., Monkey flowers; *Nasonia* spp., Wasps.

^dInvolves four species of *Mimulus*. Values reported are averages over six species crosses.

^ePublished before Ref. 6, but missed in that review.

Box 1. Power and limitation of QTL studies

The power of Quantitative Trait Locus (QTL) analyses to detect genes varies with many features of experimental design^{a-c}. Among the most important are trait heritability, marker density and mapping algorithm (e.g. interval versus composite interval versus multiple interval mapping). Tradeoffs also occur among algorithms; statistical power can, for instance, be diverted from QTL detection to QTL localization^a. But the most important determinant of power is F2 and/or backcross sample size.

Unfortunately, small sample sizes not only reduce power to find QTL, but also affect estimates of QTL effects. Worse, this effect is biased: small sample sizes lead to systematic overestimation of QTL additive effects, the so-called 'BEAVIS EFFECT' (see Glossary)^{c,d}. The cause of this effect is simple. The same data are used to both detect QTL and estimate their effects. Thus, given a normal distribution of error about a true QTL effect, errors involving

overestimation yield significant QTL more often than do those involving underestimation. Fortunately, simulations suggest that this effect becomes small as experimental sample sizes near ~500 genotyped and phenotyped individuals^{c,d}.

Even with large sample sizes, experiments will not detect all true QTL, especially those of small effect. Otto and Jones^e recently showed how this problem can be partly corrected. If one knows the detection threshold below which QTL cannot be found and something about the underlying distribution of QTL effects, the number of true QTL can be back calculated from the number seen. If, for example, QTL effects are exponentially distributed^f, the maximum likelihood estimate of true QTL number is $n_{QTL} = D/(M - \theta)$, where D is half the phenotypic difference between parental lines, M is the mean additive effect among detected QTL, and θ is the threshold below which QTL cannot be detected. Otto and

Jones show that their method accurately estimates true QTL number in simulations that conform to their assumptions.

References

- a Basten, C.J. *et al.* (1996) *A Reference Manual and Tutorial for QTL Mapping*, North Carolina State University
- b Kearsey, M.J. and Farquhar, G.L. (1998) QTL analysis in plants; where are we now? *Heredity* 80, 137–142
- c Beavis, W.D. (1998) QTL analyses: power, precision, and accuracy. In *Molecular Dissection of Complex Traits* (Paterson, A.H., ed.), pp. 145–162, CRC Press
- d Beavis, W.D. (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. In *Proceedings of the Corn and Sorghum Industry Research Conference*, pp. 250–266, American Seed Trade Association
- e Otto, S.P. and Jones, C.D. (2000) Detecting the undetected: estimating the total number of loci underlying a trait in QTL analyses. *Genetics* 156, 2093–2107
- f Orr, H.A. (1998) The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution* 52, 935–949

showed that a difference in larval morphology (presence or absence of a fine lawn of hairs on the dorsal surface of abdominal segments) between *Drosophila simulans* and *Drosophila sechellia* is the result of a single gene. This gene, *ovo/shaven-baby*, was identified through a combination of deficiency mapping and complementation tests and further results suggest, although not prove, that the species difference reflects divergence in *cis* regulation at the gene.

Second, Jones⁹ showed that another trait difference (adult resistance to a host plant toxin) between the same species pair has an oligogenic basis, with at least five factors involved. Repeated tests with large sample sizes further showed that several large chromosome regions have no effect on toxin resistance, suggesting that the total number of genes involved is modest. Finally, Zeng *et al.*¹⁰ showed that a difference between *Drosophila simulans* and *Drosophila mauritiana* in the size/shape of the posterior lobe of the male genital arch involves at least 19 QTL.

Such results do not encourage the idea that the genetics of species differences shows any regularities. And there is, of course, no fundamental reason why the number of factors should show any consistency; every case of divergence might differ from every other, reflecting differences in the strength of selection, the nature of the standing genetic variation, and so on. It is at least possible, however, that the variation seen in Table 1 masks real regularities in the underlying number of genes. This masking could be a result of either (1) problems

of resolution; or (2) the too-casual pooling of results across what are actually different classes of evolutionary events.

There is no doubt that different studies have different power to find genes. Whereas biometric studies, in particular, are notoriously bad at estimating gene number, QTL studies also vary enormously in power (Box 1). This effect of power can be seen by comparing Liu *et al.*'s¹¹ early analysis of posterior lobe differences in *Drosophila*, which found only eight QTL, with Zeng *et al.*'s¹⁰ later and larger study, which found 14 (via composite interval mapping; 19 were found via multiple interval mapping; for another example, see Bradshaw *et al.*^{12,13}). Despite this, it is unlikely that power differences can explain some of the most interesting differences in Table 1. Note, for example, that some of the most rigorous studies, such as those using deficiency mapping in which chromosome breakpoints are known with precision⁸, uncover the fewest factors. Similarly, in several cases in which few factors were found, for example, carotenoid concentration in *Mimulus*¹³, detected QTL explain essentially all of the F2 phenotypic variance, suggesting that few factors of substantial effect were missed. Finally, some species differences simply MENDELIZE^{6,14}.

The cases shown in Table 1 could, however, differ from each other in a systematic and biologically interesting way. There are two obvious possibilities. The first is that the species pairs shown might have diverged for different amounts of time; all else being equal, 'older' taxa would be expected to differ by more factors than do younger ones. It is important to note

that this remains true even if directional selection in old versus young species pairs acted for the same length of time because, under stabilizing selection, characters will show 'genetic turnover' such that the genes affecting a trait will continue to diverge even after trait means have stopped changing^{15,16}. (This argument suggests, incidentally, that analysis of two species that do not differ at a trait should uncover QTL, although necessarily a mix of plus and minus factors in each species.) Although differences in divergence time surely contribute to variation in gene number, they cannot explain the whole picture. Indeed, the three *Drosophila* cases described above (one monogenic, one oligogenic and one polygenic) all involve the same species pair (*D. simulans*–*D. sechellia* or, nearly equivalently, *D. simulans*–*D. mauritiana*). Normalizing QTL number by divergence time would not eliminate these differences.

Finally, different characters might have experienced qualitatively different kinds of selection. Orr and Coyne⁶ suggested, for instance, that the genetic bases of response to natural versus SEXUAL SELECTION might differ systematically. Natural selection might sometimes involve a single bout of adaptation to a new optimum, whereas sexual selection might typically involve repeated bouts of coevolution between male and female traits, yielding more polygenic bases. Although it would be absurd to claim that we have good support for this idea, it is worth noting that, among QTL studies, the two most polygenic results in Table 1 (those of 19 QTL in Ref. 10 and of 11 QTL in Ref. 17) both involve divergence in male genitalia, a character that is rapidly evolving in many insects and that is surely strongly sexually selected¹⁸. This seems a promising avenue for future work: do species differences that reflect natural versus sexual selection differ consistently in QTL number? It is possible that this represents another source of confusion, a failure to distinguish between what are fundamentally different kinds of species differences.

Major genes

We would like to know something about the distribution of phenotypic effects among the genes underlying species differences; indeed this question seems far more interesting than ones about total gene number. Whereas theory suggests that the distribution of gene effects under selection should be nearly exponential (Box 2), present data on species differences are far too rough to allow meaningful tests of this prediction (response to artificial selection in the laboratory, however, does appear to involve exponential effects, as predicted⁷). We turn, therefore, to a more qualitative question: do genes of large effect play a role in response to selection?

Major genes clearly play a role in responses to artificial selection and human disturbance. Indeed, this represents the most important finding of recent

QTL studies. Many cases are now known, for instance, in which major genes played a key role in shaping crop plant morphology^{19–22}. In several of these cases (e.g. *tb1*, which affects lateral branching pattern in maize²³, and *fw2.2*, which affects fruit size in tomato²⁴) the relevant gene has been molecularly identified and characterized, weighing against the notion that major QTL correspond to many closely linked genes having individually small effects. (These results do not, however, rule out the subtler possibility that the effect of a single QTL is the result of several QTN, i.e. to several nucleotide substitutions within the same gene or regulatory region.) Similar results have been obtained in studies of response to selection caused by human disturbance, such as resistance to the insecticide cyclodiene, which is based on the same amino acid substitution in the same gene (*Rdl*, resistance to dieldrin) in all insect orders studied²⁵. Major genes sometimes also play a role in natural phenotypic differences within species^{26–29}.

Historically, however, the most contentious question has concerned whether major genes play a role in species differences. It is now clear that the answer is yes, sometimes. Sucena and Stern's work⁸ shows that a single gene explains morphological divergence at a larval trait distinguishing two *Drosophila* spp. Similarly, Takahashi *et al.*³⁰ recently found that an insertion/deletion difference at a single gene apparently completely explains the difference in cuticular hydrocarbon profile between African/Caribbean and cosmopolitan 'RACES' of *D. melanogaster*. Outside of *Drosophila*, evidence for major factors has been found between species of a wasp, *Nasonia*, where 44% of a large (2.4-fold) difference in wing size maps to a single chromosome region³¹. But, perhaps the best known case of major QTL effects involves morphological differences between two species of the monkeyflower, *Mimulus lewisii* and *Mimulus cardinalis*. Although one to six QTL underlie each of the floral traits studied, Bradshaw *et al.*¹³ showed that nine of 12 traits involve at least one QTL explaining >25% of the species difference. Indeed, a single QTL at the *yup* locus qualitatively affects carotenoid concentration, explaining 83% of the phenotypic variance among F2 hybrids.

Such results are not, however, universal. In their study of genital arch divergence in *Drosophila*, for instance, Zeng *et al.*¹⁰ found that the largest QTL explains only 11% of the species difference; most QTL had considerably smaller effects. Similarly, Lin and Ritland³² suggested that morphological differences affecting selfing rate differences between two species of *Mimulus* are controlled by many loci, most of small effect; Fenster and Ritland's³³ biometric analysis of morphological differences between several *Mimulus* spp. arrived at a similar conclusion.

These results are, at face value, inconsistent with major gene effects. But as True *et al.*³⁴ emphasized,

Box 2. Distribution of gene effects

Evolutionary theory has had little to say about the expected distribution of phenotypic effects among alleles fixed during response to selection. Although central to Quantitative Trait Locus (QTL) and experimental evolution work, the problem appears to fall between the two great theoretical traditions in evolutionary biology, population and quantitative genetics, at least as typically pursued. Population genetics speaks in terms of fitness, rather than phenotypic effects, whereas quantitative genetics rests largely (although not entirely) on an infinitesimal foundation in which response to selection involves a nearly infinite number of loci of nearly infinitesimally small effect each. Theoretical treatment of the expected distribution of gene effects obviously requires analysis of an alternative model in which different mutations are allowed different-sized phenotypic effects.

Fisher proposed such a model in 1930 (Ref. a). His geometric model of adaptation considers evolution in a high dimensional phenotypic space, in which each dimension represents a character. The local optimal combination of trait values sits at some point in space (e.g. the origin), whereas the population presently sits at some other point, off the optimum. The population attempts to evolve towards the optimum by producing random mutations in phenotypic space, where mutations are vectors having different magnitudes. Although most mutations are deleterious, a few are favorable (point to the optimum) and so enjoy positive probabilities of fixation^b. Response to selection can thus be pictured as an adaptive walk to the optimum, involving the sequential substitution of favorable mutations, each potentially having a different phenotypic size. Recent theory shows that such adaptive walks are characterized by substitution of factors having exponentially distributed phenotypic effects^c (Fig. 1): response to selection involves many factors of small effect and a few of large effect, as argued by Robertson^d. Further work shows that this exponential distribution is robust to many of the assumptions of the model (such as shape of the fitness function and the exact distribution of mutational effects)^{e,e}. Exponential distributions of effects appear to be a fairly general property of adaptation to a fixed optimum, at least when evolution involves new or rare mutations.

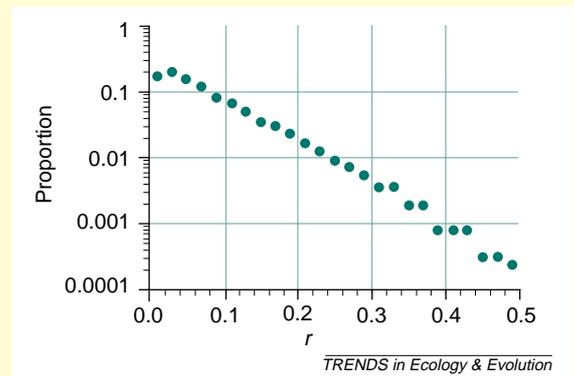


Fig. 1. Distribution of phenotypic effects, r , among genes fixed during response to selection, where evolution occurred in Fisher's geometric model. The distribution is approximately exponential (a straight line on a semi-log plot). The simulation results shown reflect many realizations of adaptive walks to the optimum in a 25-dimensional (character) phenotypic space. Response to selection involved fixation of new mutations. For details, see Refs c,e.

QTL data not only reveal the magnitude of QTL effect, but also their sign. Interestingly, QTL studies often uncover a mixture of 'plus' and 'minus' factors in the high line^f (a tall line of plants might, for example, carry some 'short' alleles). The concentration of plus factors in the high line provides a test of the role of natural selection in trait divergence. In particular, one can ask if the high line carries more plus alleles than expected under the null hypothesis of neutral evolution, conditioning on the known phenotypic difference between parental lines^g. In several cases, this QTL sign test has allowed rejection of neutrality, yielding evidence for the role of directional selection in trait divergence^g.

References

- a Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*, Oxford University Press
- b Kimura, M. (1983) *The Neutral Theory of Molecular Evolution*, Cambridge University Press
- c Orr, H.A. (1998) The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution* 52, 935–949
- d Robertson, A. (1967) The nature of quantitative genetic variation. In *Heritage from Mendel* (Brink, A., ed.), pp. 265–280, The University of Wisconsin Press
- e Orr, H.A. (1999) The evolutionary genetics of adaptation: a simulation study. *Genet. Res.* 74, 207–214
- f Tanksley, S.D. (1993) Mapping polygenes. *Annu. Rev. Genet.* 27, 205–233
- g Orr, H.A. (1998) Testing natural selection versus genetic drift in phenotypic evolution using Quantitative Trait Locus data. *Genetics* 149, 2099–2104

this conclusion neglects a fundamental ambiguity in the meaning of magnitude of phenotypic effect, another of our confusions. A gene effect might be large because a factor explains a substantial fraction of the phenotypic gap between species (or, similarly, of the F₂ phenotypic variance), or because it has a substantial effect relative to the standing phenotypic

variation within species. These definitions are not equivalent. If two species differ dramatically in a trait, say by 100 phenotypic standard deviations (σ), a factor of 5σ effect explains only one-twentieth of the species gap but has enormous effect relative to the standing variation. Less obviously, if two species differ by a single QTL explaining a 0.1σ difference in

some trait, the QTL is large in that it explains 100% of the species gap, but is small relative to the standing variation. The preference of QTL workers for 'large' in the species gap sense is surely an artifact of their penchant for analyzing dramatic species differences.

There would seem, however, two good reasons for measuring QTL effect relative to standing variation. The first is that this is the size that natural selection sees. When a new mutation appears, natural selection cannot know the fraction of the final species gap that the factor will ultimately explain, a figure that depends, among other things, on the number of environmental changes that ultimately occur. Second, phenotypic size in the standing variation sense is roughly time invariant, whereas that in the species gap sense is not. The first mutation fixed explains 100% of the species gap as measured immediately after its substitution, but less and less as the analysis is performed later and later.

True *et al.*'s distinction qualitatively affects our interpretation of the literature on species differences. To see this, note that the data that superficially appear to provide the best evidence against major genes (Zeng *et al.*'s findings on genital arch morphology) actually provide strong evidence for them by the standing variation criterion, as Zeng *et al.* themselves emphasize. Because genital arch size and shape differs by 35 phenotypic standard deviations between *D. simulans* and *D. mauritiana*, the largest QTL detected has a massive effect of 4σ , while seven others have effects of $>2\sigma$. (These effects are, however, inflated somewhat, as highly inbred lines were used and effects are therefore measured in environmental phenotypic standard deviations.)

Relative to the variation seen within species, it is doubtful that any study of species differences has failed to find major QTL. One possible implication of this finding is that species differences might often be built from new (or at least rare) mutations, rather than from polygenes segregating at appreciable frequencies (although factors of $\sim 1\sigma$ effect might segregate at low frequencies). The notion that the genetic stuff of species differences is not identical to the quantitative genetic variation within species also finds some support from the finding that an appreciable fraction of phenotypic standing variation is caused by large insertions (presumably transposable elements), which are presumably unconditionally deleterious and so do not often contribute to fixed species differences (e.g. see Mackay and Langley's³⁵ and Long *et al.*'s³⁶ analyses of molecular variation at the *achaete-scute* complex and its contribution to bristle number variation in *Drosophila*).

The role of epistasis

The genetic basis of species differences might be complex in another way. It might involve complicated NETWORKS of epistatic interactions. Although epistatic interactions appear to be fairly common within

species^{7,37}, earlier surveys concluded that QTL between long-diverged taxa generally show little epistasis⁵. On the whole, this conclusion still stands. Among recent studies, for instance, two of the largest^{9,10} both noted the small role of epistasis. This is not to say, of course, that epistasis plays no role in species differences. Bradshaw *et al.*¹³, for instance, found some evidence for epistasis among the factors underlying carotenoid concentration in *Mimulus*, whereas True *et al.*³⁴ found similar evidence among factors underlying anal plate bristle number in *Drosophila*. Strong epistasis would, however, generally appear to be the exception.

Although new mapping procedures, for example, multiple interval mapping³⁸, might increase our ability to detect epistasis and reach firmer conclusions, it is worth asking why evolutionists are so interested in epistasis among the genes causing species differences in the first place. The answer is evidently that the genetics of species differences appear to tell us something about the processes involved in the origin of these differences. But, in the case of epistasis, this is not necessarily true. The reason reflects another confusion – the epistasis seen in hybrids must be distinguished from that which appeared during the evolution of the parental species. The two are not equivalent because experimental hybrid backcrosses or F2 generations include genotypes that never segregated within either species. This is seen most easily if one imagines that the relevant substitutions in each LINEAGE occurred sequentially, with each mutation appearing and sweeping to fixation on an essentially homogeneous background. Although the identity of alleles fixed in earlier substitutions might well constrain those that can get fixed in later substitutions, all alleles are selected solely for their additive effects, with no possible role for epistasis. This does not, however, preclude the possibility that epistasis might be strong among experimental hybrids, a point that Coyne *et al.*³⁹, among others, have emphasized.

Although the problem of inferring the forces that acted during evolution from the genetics of present species differences is not limited to epistasis, it seems most acute here. When considering the number of genes involved, for instance, the observed number of QTL must be less than or equal to the true number. But no such relationship holds for epistasis. The proportion of pairwise combinations of genes showing epistasis in analyses of species differences might be greater than, less than, or equal to the proportion that showed such interactions during evolution. Strictly speaking, the only result that can be interpreted with confidence is perfect additivity: if all possible gene combinations in hybrids show purely additive action, we can safely conclude that epistasis played no role in divergence. [Note that the other extreme (all pairs showing epistasis) does not guarantee that epistasis was expressed during

evolution, as shown by our sequential example.] In general, we do not know how to infer the role of epistasis in trait evolution from genetic data on species differences.

It should also be noted that the evolutionary importance of epistasis (even if expressed during evolution) is sometimes less than clear. For instance, although most studies focus on the detection of significant QTL interactions, such effects might have played an inconsequential role in adaptive evolution if the genes involved had substantial additive effects in the desired direction.

Species differences versus reproductive isolation

It is worth comparing what is known about the genetics of species differences with what is known about the genetics of reproductive isolation. The genetics of PREZYGOTIC ISOLATION (i.e. of barriers that prevent hybrid formation) can clearly resemble that of ordinary differences. The reason is that prezygotic isolation is often caused by differences in ordinary traits, such as pheromone or courtship song (Table 1). For the same reason, the genetics of EXTRINSIC POSTZYGOTIC ISOLATION, in which hybrids have intermediate phenotypes that fail to fit available niches, must also resemble that of ordinary species differences – such isolation is again caused by ordinary differences.

The basis of intrinsic postzygotic isolation (hybrid sterility and inviability), however, differs profoundly from that of ordinary species differences. For one thing, hybrid sterility and inviability involve ubiquitous and strong epistasis. This epistasis involves both interactions between alleles (at different loci) from different species and interactions between alleles (at different loci) from the same species. The first kind of interaction is required for the evolution of intrinsic postzygotic isolation, as emphasized by the Dobzhansky–Muller model of speciation (any allele that causes sterility or inviability on a foreign genetic background must not do so on its normal genetic background^{40–42}). The second kind of interaction is also expected on theoretical grounds, because conspecific epistasis increases the fraction of evolutionary paths leading to the present species that do not pass through a sterile or inviable genotype⁴². In any case, postzygotic isolation clearly involves far stronger epistasis among experimental hybrids than do ordinary species differences. Given that experimental analyses of postzygotic isolation and ordinary species differences largely proceed in the same way (indeed, they are sometimes performed in the same study¹⁷), this disparity cannot be the result of differences in the statistical power to detect interactions. It is important to appreciate, however, that, even in the case of intrinsic postzygotic isolation, the strong epistasis seen in experimental hybrids implies little or nothing about interactions among

genes during evolution. Once again, divergence could have conceivably involved sequential substitution of mutations with no possible role for epistasis.

Second, alleles causing intrinsic postzygotic isolation tend to act recessively in hybrids, a pattern that helps explain HALDANE'S RULE and the disproportionate effect of the X chromosome on hybrid sterility and inviability (reviewed in Ref. 43; the biochemical basis of this recessivity remains unresolved). No such bias to recessivity is seen among the genes underlying ordinary morphological or behavioral differences, either within or between species; instead roughly additive effects appear common^{5,44}. As expected, therefore, the X chromosome does not play a disproportionately large role in ordinary species differences; rather, QTL appear fairly evenly spread over the genome⁴⁵. (Curiously, there is an exception. For reasons that remain unclear, phenotypic differences within and between lepidopteran species often involve the X chromosome⁴⁶.)

It appears, therefore, that characters that are not normally expressed within species (hybrid sterility and inviability) show strong epistasis and recessivity in hybrids, whereas those that are normally expressed within species (ordinary species differences) do not. It will be interesting to see if a breakdown in 'normal' characters in hybrids (for example, the failure of certain bristles to develop in *Drosophila* hybrids) behaves genetically more like postzygotic isolation or like ordinary morphological species differences. Such phenotypes would certainly be expected to behave in a similar way to postzygotic isolation.

Conclusions

Although we cannot yet make many generalizations about the genetics of species differences, a few points seem clear. First, the number of QTL underlying species differences is highly variable. In some cases, these differences are real, not artifacts of differences in statistical power. Second, major genes sometimes play a role in the evolution of species differences. Indeed, in several cases, the relevant gene has been identified molecularly. Finally, epistasis and recessivity are far rarer and weaker for ordinary species differences than they are for hybrid sterility and inviability.

Our difficulties in reaching firm conclusions about the genetics of species differences reflect several problems: a shortage of data; the imperfect resolution of genetic analyses; and perhaps as important, a set of conceptual confusions that compromise the interpretation of the data that we do have. I have noted several of these confusions here. Although keeping these distinctions straight will not render the genetics of species differences suddenly clear, it seems a necessary step towards that goal.

Acknowledgements

I thank N. Barton, R. Butlin, J. Coyne, C. Jones, L. Rieseberg and M. Turelli for helpful comments and discussion. This work was supported in part by National Institutes of Health grant GM51932 and by the David and Lucile Packard Foundation.

References

- 1 Rundle, H.D. *et al.* (2000) Natural selection and parallel speciation in sympatric sticklebacks. *Science* 287, 306–308
- 2 Futuyma, D.J. (1987) On the role of species in anagenesis. *Am. Nat.* 130, 465–473
- 3 Haldane, J.B.S. (1938) The nature of interspecific differences. In *Evolution* (de Beer, G.R., ed.), pp. 19–94, Clarendon Press
- 4 Ford, E.B. (1964) *Ecological Genetics*, Methuen
- 5 Tanksley, S.D. (1993) Mapping polygenes. *Annu. Rev. Genet.* 27, 205–233
- 6 Orr, H.A. and Coyne, J.A. (1992) The genetics of adaptation revisited. *Am. Nat.* 140, 725–742
- 7 Mackay, T.F.C. (2001) Quantitative trait loci in *Drosophila*. *Nat. Rev. Genet.* 2, 11–20
- 8 Sucena, E. and Stern, D.L. (2000) Divergence of larval morphology between *Drosophila sechellia* and its sibling species caused by cis-regulatory evolution of *ovdshaven-baby*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4530–4534
- 9 Jones, C.D. (1998) The genetic basis of *Drosophila sechellia*'s resistance to a host plant toxin. *Genetics* 149, 1899–1908
- 10 Zeng, Z.-B. *et al.* (2000) Genetic architecture of a morphological shape difference between two *Drosophila* species. *Genetics* 154, 299–310
- 11 Liu, J. *et al.* (1996) Genetic analysis of a morphological shape difference in the male genitalia of *Drosophila simulans* and *D. mauritiana*. *Genetics* 142, 1129–1145
- 12 Bradshaw, H.D. *et al.* (1995) Genetic mapping of floral traits associated with reproductive isolation in monkey flowers (*Mimulus*). *Nature* 376, 762–765
- 13 Bradshaw, H.D. *et al.* (1998) Quantitative trait loci affecting differences in floral morphology between two species of monkey flower (*Mimulus*). *Genetics* 149, 367–382
- 14 Whitaker, T.W. (1944) The inheritance of certain characters in a cross between two American species of *Lactuca*. *Bull. Torrey Bot. Club* 71, 347–355
- 15 Lande, R. (1975) The maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genet. Res.* 26, 221–235
- 16 Barton, N.H. (1989) The divergence of a polygenic system under stabilising selection, mutation and drift. *Genet. Res.* 54, 59–77
- 17 Macdonald, S.J. and Goldstein, D.B. (1999) A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. *Genetics* 153, 1683–1699
- 18 Eberhard, W.G. (1985) *Sexual Selection and Animal Genitalia*, Harvard University Press
- 19 Dorweiler, J. *et al.* (1993) Teosinte glume architecture 1: a genetic locus controlling a key step in maize evolution. *Science* 262, 233–235
- 20 Doebley, J. *et al.* (1994) Inheritance of kernel weight in two maize-teosinte hybrid populations: implications for crop evolution. *J. Hered.* 85, 191–195
- 21 Fatokun, C.A. *et al.* (1992) Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. *Genetics* 132, 841–846
- 22 Grandillo, S. and Tanksley, S.D. (1996) QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium*. *Theor. Appl. Genet.* 92, 935–951
- 23 Wang, R.-L. *et al.* (1999) The limits of selection during maize domestication. *Nature* 398, 236–239
- 24 Frary, A. *et al.* (2000) *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. *Science* 289, 85–88
- 25 French-constant, R.H. (1994) The molecular and population genetics of cyclodiene insecticide resistance. *Insect Biochem. Mol. Biol.* 24, 335–345
- 26 Cubas, P. *et al.* (1999) An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401, 157–161
- 27 Smith, T.B. (1993) Disruptive selection and the genetic basis of bill size polymorphism in the African finch *Pyrenestes*. *Nature* 363, 618–620
- 28 Grant, M.R. *et al.* (1998) Independent deletions of a pathogen-resistance gene in *Brassica* and *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15843–15848
- 29 van Dam, N.M. *et al.* (1999) Inheritance and distribution of trichome phenotypes in *Datura wrightii*. *J. Hered.* 90, 220–227
- 30 Takahashi, A. *et al.* The nucleotide changes governing pheromonal variation in *Drosophila* and their evolution. *Proc. Natl. Acad. Sci. U. S. A.* (in press)
- 31 Weston, R.F. *et al.* (1999) Genetics of a wing size difference between two *Nasonia* species. *J. Evol. Biol.* 12, 586–595
- 32 Lin, J.-Z. and Ritland, K. (1997) Quantitative trait loci differentiating the outbreeding *Mimulus guttatus* from the inbreeding *M. platycalyx*. *Genetics* 146, 1115–1121
- 33 Fenster, C.B. and Ritland, K. (1994) Quantitative genetics of mating system divergence in the yellow monkeyflower species complex. *Heredity* 73, 422–435
- 34 True, J.R. *et al.* (1997) Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *D. mauritiana*. *Evolution* 51, 816–832
- 35 Mackay, T.F. and Langley, C.H. (1990) Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*. *Nature* 348, 64–66
- 36 Long, A.D. *et al.* (2000) Both naturally occurring insertions of transposable elements and intermediate frequency polymorphisms at the *achaete-scute* complex are associated with variation in bristle number in *Drosophila melanogaster*. *Genetics* 154, 1255–1269
- 37 Long, A.D. *et al.* (1995) High resolution mapping of genetic factors affecting abdominal bristle number in *Drosophila melanogaster*. *Genetics* 139, 1273–1291
- 38 Kao, C.-H. *et al.* (1999) Multiple interval mapping for quantitative trait loci. *Genetics* 152, 1203–1216
- 39 Coyne, J.A. *et al.* (1997) A critique of Sewall Wright's shifting balance theory of evolution. *Evolution* 51, 643–671
- 40 Dobzhansky, T. (1937) *Genetics and the Origin of Species*, Columbia University Press
- 41 Muller, H.J. (1942) Isolating mechanisms, evolution, and temperature. *Biol. Symp.* 6, 71–125
- 42 Orr, H.A. (1995) The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics* 139, 1805–1813
- 43 Turelli, M. and Orr, H.A. (2000) Dominance, epistasis and the genetics of postzygotic isolation. *Genetics* 154, 1663–1679
- 44 Kearsey, M.J. and Farquhar, G.L. (1998) QTL analysis in plants; where are we now? *Heredity* 80, 137–142
- 45 Coyne, J.A. and Orr, H.A. (1989) Two rules of speciation. In *Speciation and its Consequences* (Otte, D. and Endler, J., eds), pp. 180–207, Sinauer Associates
- 46 Prowell, D.P. (1998) Sex linkage and speciation in Lepidoptera. In *Endless Forms: Species and Speciation* (Howard, D.J. and Berlocher, S.H., eds), pp. 303–319, Oxford University Press
- 47 Jones, C.D. The genetic basis of larval resistance to a host plant toxin in *Drosophila sechellia*. *Genet. Res.* (in press)
- 48 Jones, C.D. The genetic basis of *Drosophila sechellia*'s oviposition site preference. *Evolution* (in press)
- 49 Coyne, J.A. (1996) Genetics of a difference in male cuticular hydrocarbons between two sibling species, *Drosophila simulans* and *D. sechellia*. *Genetics* 143, 1689–1698
- 50 Coyne, J.A. and Charlesworth, B. (1997) Genetics of a pheromonal difference affecting sexual isolation between *Drosophila mauritiana* and *D. sechellia*. *Genetics* 145, 1015–1030
- 51 Coyne, J.A. (1996) Genetics of differences in pheromonal hydrocarbons between *Drosophila melanogaster* and *D. simulans*. *Genetics* 143, 353–364
- 52 Tomaru, M. and Oguma, Y. (1994) Genetic basis and evolution of species-specific courtship song in the *Drosophila auraria* complex. *Genet. Res.* 63, 11–17
- 53 Shaw, K.L. (1996) Polygenic inheritance of a behavioral phenotype: interspecific genetics of song in the Hawaiian cricket genus *Laupala*. *Evolution* 50, 256–266
- 54 Vlot, E.C. *et al.* (1992) Genetic and nongenetic factors influencing deviations from five pappus parts in a hybrid between *Microseris douglasii* and *M. bigelovii*. *Int. J. Plant Sci.* 153, 89–97
- 55 Fenster, C.B. *et al.* (1995) The genetics of floral development differentiating two species of *Mimulus* (Sclerophylariaceae). *Heredity* 74, 258–266

Do you want to reproduce material from TREE?

Except as outlined in Terms and Conditions (see p. iv), no part of *Trends in Ecology and Evolution* may be reproduced, either in print or in electronic form, without written permission from Elsevier Science.

Please send any permission requests to:

Elsevier Science Global Rights
Department
PO Box 800
Oxford
UK OX5 1DX
e-mail: permissions@elsevier.co.uk