NEWS AND COMMENTARY

Evolutionary genetics

Gene replacement and the genetics of speciation

MG Ritchie and MAF Noor

Heredity (2004) 93, 1–2, advance online publication, 14 April 2004; doi:10.1038/sj.hdy.6800456

Understanding the process of speciation remains a major challenge in evolutionary biology. Studying genes that confer reproductive isolation between sexual species is a very active but frustratingly difficult area of this research. Identifying genes of large effect in speciation is tricky, and there are probably fewer than a dozen proposed 'speciation genes', with varying degrees of credibility to their claims (Swanson and Vacquier, 2002; Noor, 2003; Wu and Ting, 2004). The ecological selection acting on alleles at these loci, and the phenotypic changes associated with gene substitution, is only beginning to be studied. So far *Drosophila* is the main organism where real progress has been made, and a recent paper by Greenberg *et al* (2003) nicely demonstrates the potential for such analyses.

The new study is possible due to two factors: a candidate gene of large effect exhibiting geographic variability; and a methodology allowing precise targeted gene replacement. The gene, *desaturase2*, is involved in the production of long-chain fatty acid hydrocarbons, which form a major component of the insect cuticle. The blend of cuticular hydrocarbons (CHCs) in species of the *Drosophila melanogaster* group is known to influence ecologically important traits, such as desiccation resistance (Rouault *et al*, 2001). However, the major component of the blend is usually sexually dimorphic, species-specific, and sometimes geographically variable. This has implicated them in sexual behaviours such as mate and species recognition (Cobb and Jallon, 1990). Their role as contact pheromones was confirmed in a series of elegant experiments by Coyne and co-workers, who demonstrated that confining a few females of, for example, *D. simulans* with abundant *D. sechellia* led to the transfer of CHCs between them. *D. simulans* males, who normally ignore *D. sechellia* females, would court them vigorously if they had been 'perfumed' with the *D. simulans* blend, and ignore conspecific females with the heterospecific perfume (Coyne *et al*,

1994). The females' own behaviour was not involved, since flash-freezing them – while tastefully adding necrophilia to an experiment on interspecific sexuality – had no effect.

D. melanogaster and *D. simulans* are geographically variable in their main CHC component, and this probably reflects the 'Out of Africa' origins of the main commensal forms of these species (Ferveur *et al*, 1996). African *D. simulans* and male *D. melanogaster* have 7-Pentacosene as the major CHC component, but most non-African strains have 7-Tricosene. *D. melanogaster* females have 5,9-Heptacosadiene (5,9-HD) in Africa, but the isomer 7,11-Heptacosadiene (7,11-HD) in non-African strains. The CHC blend has changed as these species left their African roots, but why? There are associations between CHC and climatic variables, implicating ecological selection; African strains are known to be more resistant to desiccation (Rouault *et al*, 2001). Caribbean flies and some other strains are African-like (Ferveur *et al*, 1996). This probably reflects more recent introductions, but selection may maintain this blend against gene flow from cosmopolitan strains. However, the key environmental factors (or behavioural consequences such as sexual selection) have not been identified.

The genetic control of these differences has been studied, and *desat2* identified as a key change. Cosegregation analyses implicated *desat2* in the change between 7,11-HD and 5,9-HD in *D. melanogaster* females (Coyne *et al*, 1999). Expression analyses (Dallerac *et al*, 2000) and association studies across numerous strains (Takahashi *et al*, 2001) confirmed this, and sequence analyses found (via Tajima tests) that the replacement of 5,9-HD by 7,11-HD was probably driven by strong selection (the Cosmopolitan allele is a loss of function allele, which presumably blocks an important stage in the conversion of 7,11-HD to 5,9-HD).

Greenberg *et al* (2003) have taken advantage of a technique for 'targeted' gene replacement in *D. melanogaster* to

study the consequences of transforming a cosmopolitan strain of *D. melanogaster* with the African allele of *desat2*. Most genetic transformation experiments have no control of the site of insertion of introduced alleles, which are usually inserted randomly by P-element transformation into a strain carrying a null allele. Instead, they used a recently developed technique whereby the introduced allele jumps into a genomic position alongside or within the wild-type allele. DNA repair then excises either the introduced or native locus, leading to precise, targeted, gene replacement. Using this approach they produced a strain of cosmopolitan *D. melanogaster* carrying a *desat2* locus originating from Zimbabwe (and control strains where the introduced allele was excised), and examined the phenotypic consequences.

First, the exciting, good news. Transformant females displayed an African-like CHC blend, so their production of 7,11-HD was blocked as in African flies (and conventional P-element transformants often failed to show any changes). Secondly, a series of laboratory studies showed that they displayed some traits thought to be associated with African-like adaptations. They were less cold-tolerant and more starvation resistant, like African strains. Thus, many of the features of the story seem confirmed – this single gene changes the CHC blend and is likely to have major ecological consequences for the flies, compatible with probable climate changes associated with expansion from Africa.

However, the experiment also brought some disappointing news. The transformed flies were, surprisingly and confusingly, *not* more desiccation resistant. Also, despite the apparent precision of the gene swapping, there was a suggestion that expression is altered. The amount of 7,11-HD was lower than in the donor strain, and while this may result from effects of other genes, it could also be a side effect of the gene replacement, as RNA expression in the transformed lines was apparently not assayed directly. Furthermore, the ecological changes were shown by both sexes, yet *desat2* is normally only expressed in females (Dallerac *et al*, 2000; Takahashi *et al*, 2001). Greenberg *et al* (2003) suggest that the lack of sex-specificity in the transformants implies that the original mutation may have been favoured for some general effect of the mutation on phospholipid production rather than the precise 7,11/5,9-HD polymorphism, but the study of

Dallerac *et al* (2000) suggested that it is not transcribed at all in males. Altered expression following the transformation may be a more simple explanation. It is disappointing if such a clever, apparently almost perfect, transformation technique retains some of the drawbacks of conventional P-element transformation. An ideal experiment would involve very many lines to look at variability associated with the technique, coupled with molecular confirmation of expression, but this would demand a very large investment.

The Coyne experiments (Coyne *et al*, 1994, 1999) clearly implicate CHC blend in sexual isolation as well as ecological adaptation. It would be extremely neat if the ecologically driven replacement of a single allele could, as an indirect consequence, alter mate recognition and therefore influence sexual isolation. Such a gene would truly deserve the title of a 'speciation gene'. Greenberg *et al* (2003) mount a case for *desat2* being such a gene, but the totality of the evidence so far does not make this wholly convincing. They speculate that *desat2* may play a role in the sexual isolation seen between flies from Zimbabwe and elsewhere. A series of papers have shown unusual, strong sexual isolation between Cosmopolitan and some Zimbabwe strains, and the origin of the African *desat2* allele used by Greenberg *et al* was Zimbabwe. However, their study does not look at mate choice or assortative mating in the transformed strains. Further, behavioural 'Zimbabweanness' correlates poorly

with general features of African strains – many strains have African characteristics, including 5,9-HD, but low sexual isolation from cosmopolitan strains (Hollocher *et al*, 1997; Colegrave *et al*, 2000) and genetic analysis of the assortative mating shows a polygenic architecture (Ting *et al*, 2001). Furthermore, although the role of CHC blend has been clearly demonstrated in interspecific mate recognition, whether the change between African and Cosmopolitan strains influences assortative mating within species is more contentious. Coyne *et al* (1999), following a similar CHC transfer protocol to that used between species, concluded that the transfer of 5,9/7,11-HD had no detectable effect on mating behaviour or sexual isolation. However, other authors have argued differently (Ferveur *et al*, 1996) and there is a covariance between assortment and the *desat2* locus amongst some lines (Fang *et al*, 2002).

Greenberg *et al*'s paper presents a very exciting demonstration of the potential for precise gene replacement to allow detailed analyses of the evolution of genes influencing speciation, but it falls short of a wholly convincing case. Other candidate genes exist, including the classic example of genetic transformation of the *Drosophila period* gene (a 'clock' gene). Over a decade ago, P-element transformation demonstrated that *D. melanogaster* can show both heterospecific activity cycles and courtship behaviours when transformed with a heterospecific *period* gene (Peterson

et al, 1988; Wheeler *et al*, 1991). Such changes have a clear potential to influence sexual isolation, but assortative mating between a pair of transformed strains has been tested for but not found (Ritchie and Kyriacou, 1994). As other candidate genes are identified and their role in adaptation is explored, it seems clear that we are on the threshold of understanding the evolution of 'speciation genes', but the threshold remains difficult to cross.

M Ritchie is at the School of Biology, University of St Andrews, St Andrews, Fife, Scotland KY16 9TH, UK and M Noor is at the Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA.

e-mails: mgr@st-and.ac.uk and mmoor@lsu.edu

Cobb M, Jallon J-M (1990). *Anim Behav* **39**: 1058–1067.

Colegrave N *et al* (2000). *J Evol Biol* **13**: 143–150.

Coyne JA, Crittenden AP, Mah K (1994). *Science* **265**: 1461–1464.

Coyne JA, Wicker-Thomas C, Jallon J-M (1999). *Genet Res* **73**: 189–203.

Dallerac R *et al* (2000). *Proc Natl Acad Sci* **97**: 9449–9454.

Fang S *et al* (2002). *Genetics* **162**: 781–784.

Ferveur J-F *et al* (1996). *Genetica* **97**: 73–80.

Greenberg AJ *et al* (2003). *Science* **302**: 1754–1757.

Hollocher H *et al* (1997). *Evolution* **51**: 1175–1181.

Noor MAF (2003). *Nature* **423**: 699–700.

Peterson G, Hall JC, Rosbash M (1988). *EMBO* **7**: 3939–3947.

Ritchie MG, Kyriacou CP (1994). *Mol Ecol* **3**: 595–599.

Rouault J, Capy P, Jallon J-M (2001). *Genetica* **110**: 117–130.

Swanson WJ, Vacquier VD (2002). *Nat Rev Genet* **3**: 137–144.

Takahashi A *et al* (2001). *Proc Natl Acad Sci USA* **98**: 3920–3925.

Ting C-T, Takahashi A, Wu C-I (2001). *Proc Natl Acad Sci USA* **98**: 6709–6713.

Wheeler DA *et al* (1991). *Science* **251**: 1082–1085.

Wu C-I, Ting C-T (2004). *Nat Rev Genet* **5**: 114–122.