Are the same genes responsible for intra- and interspecific variability for sex comb tooth number in *Drosophila*?

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The identification of genes contributing to speciation has the potential to provide important insights into the mechanisms of evolution. One of the most interesting unresolved puzzles is the relationship between intraspecific variability in morphological traits and their interspecific divergence. Intraspecific polymorphisms without major detrimental side-effects may serve as a substrate for selection response during speciation. The same quantitative trait loci (QTLs) may, then, account for the trait variability both within and between species. In contrast, the vast majority of intraspecific variants could be deleterious mutations that have not yet been selected out. In this case intraspecific variation would not ultimately generate interspecific trait differences. In previous work, QTLs responsible for morphological differences between animal species, including those for the secondary sexual trait sex comb tooth number, have been mapped with the resolution of chromosome segments. Here, we mapped QTLs for which alleles segregated between two laboratory lines of *Drosophila melanogaster*. The two QTLs identified mapped to the X chromosome and accounted for only 8% of the between-line differences, implying that a large number of small-effect genes modify sex combs. One intraspecific QTL mapped to the same interval as the QTL for interspecific differences between *D. simulans* and *D. mauritiana*. Whether or not these effects result from the same genes requires further examination.

Keywords: *Drosophila*, QTL, sex comb.

Introduction

In the last decade, there has been renewed interest in the genetics of speciation, yielding a number of new models and empirical results. Three major directions have emerged. The first direction deals with the causes of speciation. It addresses the question how a species can split into isolated lineages as it diversifies (Kondrashov & Kondrashov, 1999). The second goal is to understand the mechanisms of morphological divergence. The question is how in principle can morphological characters and behaviour be changed (see for example Rogers *et al.*, 1995). The third direction is to infer actual genetics of the differences between species. The question addressed is how do genes change during and after speciation (see for review Coyne & Orr, 1998). Ideally, these directions will merge together when it is understood which changes in gene expression lead to modifications of biochemical and developmental pathways during speciation.

Most experimental data have so far been obtained about genes responsible for interspecific postzygotic isolation. For morphological and behavioural differences between species, however, chromosomes or their segments were identified in far fewer studies (a total of 38 studies are reviewed by Coyne & Orr, 1998) and resolution at the gene level has not been achieved. In contrast, developmental biologists have found many genes which, when mutated, change morphology of the traits exhibiting interspecific differences (see for instance Carroll, 1995). The role of these genes in causing morphological differences during speciation has not yet been tested directly. The identification of genes responsible for interspecific differences in morphology has, thus, the potential to provide important insights into the mechanisms of morphological divergence attained during speciation (True *et al.*, 1997). Sex comb tooth number is a good trait for such a study for several reasons.

Sex combs are a secondary sexual trait in *Sophophora*. Spieth (1952) observed a relationship between the behaviour used by males of different species attempting copulation and the presence of sex combs.

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Males with sex combs attempt intromission before mounting is complete, but males of species without sex combs do not. Using this distinction, Spieth speculated that the combs are necessary in precopulatory manoeuvring by males. In *Drosophila melanogaster*, sex combs are used by males during both courtship and mounting. Copulatory success for males whose sex combs were surgically removed was reduced (Spieth, 1952; Coyne, 1985). Selection on tooth number has also been documented in nature. Mating wild *D. simulans* males had significantly fewer teeth than did males not found copulating (Markow *et al.*, 1996). Because the number of sex comb teeth affects mating, and the preferences of females for this structure may vary, the number of teeth and their positioning are perhaps under sexual selection. This selection should cause rapid changes in sex comb morphology and correlated changes in mating behaviour (Carson & Lander, 1984). Supporting this view, sex combs strongly differ between races and species in the number of teeth per row, in the number of rows, and in the positions and orientation of rows. This makes sex comb structure one of the most reliable and precise keys for species classification.

The genetics of sex combs has been studied in species of the *D. melanogaster* complex. Sex combs are comprised of a single row of teeth in *D. simulans* and *D. mauritiana* with mean tooth numbers of 10.12 and 13.93, respectively. Coyne (1985) showed that this interspecific difference is coded by at least five quantitative trait loci (QTLs), a minimum of one on the X chromosome and one on both arms of each large autosome. The refined analysis of True *et al.* (1997) revealed two QTLs on the third chromosome. The first QTL mapped between markers *tra* (cytological position 73A on the *D. melanogaster* map) and *Antp* (84BD); the second mapped between *Ald* (97B) and *janA* (99D) on the third chromosome. The first QTL explained +53.6%, and the second QTL -21.5% of the interspecific difference (+ stands for right, and - stands for wrong QTL effects in comparison with the direction of interspecific differences). Two additional weaker QTLs were suggested, mapping to the interval between markers *v* (10A) and *run* (19E) of the X chromosome, and near the marker *twi* (59C) of the second chromosome. The interspecific difference in sex comb is, thus, controlled by a genetically tractable number of QTLs (True *et al.*, 1997).

Sex comb tooth number varies within species. In different lines of *D. melanogaster*, sex comb tooth number was 10.17, 10.69, 10.72, and 10.73 (Choi *et al.*, 1994) with a significant line component of variance ($P < 0.0004$, our reanalysis). These intra-specific polymorphisms may have served as a substrate

for the selection response during speciation. If this hypothesis is correct, the same polymorphisms should be responsible for the trait variability both within and between species. Alternatively, the vast majority of intraspecific variants could be deleterious mutations that have not yet been selected out. In this case intraspecific variation does not ultimately generate interspecific trait difference during speciation. No data are available about the genetics of intraspecific variability in the sex comb tooth number. Here, we identify QTLs responsible for intraspecific variability in the trait and compare them with the QTLs responsible for interspecific differences.

Materials and methods

The number of sex comb teeth was scored on each foreleg of 40 males of Oregon R (Lindsley & Zimm, 1992), 2b (Pasyukova & Nuzhdin, 1993), and 98 recombinant inbred (RI) lines derived from these parental lines. Legs were mounted on tape and teeth were counted on the monitor image from a Zeiss Axioplan 2 microscope (the microscope magnification was 100, with another 2–7 magnification from the optovar device connected between the microscope and the camera).

The origin and genotyping of RI lines was described by Nuzhdin *et al.* (1997). Briefly, the F_1 progeny of the parental lines were backcrossed to 2b, and the backcrossed progeny were randomly mated for four generations. At generation five, 200 individual pairs were selected, and their progeny were inbred by full-sib mating for 25 generations. The genetic constitution of each RI line was previously determined by the analysis of 92 *roo* TE polymorphic markers that are fixed within the parental lines but segregate between them. The markers were in 1B, 3E, 4F, 5D, 6E, 7D, 7E, 9A, 10D, 11C, 11D, 12E, 14C, 15A, 15B, 15F, 16A, 16D, 17C, 18A, 19A, 19C, 21E, 22F, 26F, 29F, 30A, 30D, 33E, 34EF, 35BC, 38A, 38E, 39A, 43A, 43E, 44C, 44F, 46A, 46C, 48D, 49D, 50B, 50D, 50F, 57C, 57D, 57F, 60E, 61A, 61D, 63A, 65A, 65D, 67D, 68B, 68C, 69D, 70C, 71E, 72A, 73D, 76A, 76B, 77A, 77E, 78D, 79C, 79E, 82D, 85A, 85F, 87B, 87E, 87F, 88E, 89B, 91A, 91D, 92A, 93A, 93B, 94D, 96A, 96F, 97D, 97E, 98A, 99A, 99B, 99E and 100A. Chromosome 4 was marked with *spa*^{pol}. The lines surviving inbreeding were maintained by mass matings of ≈ 20 pairs for ≈ 40 generations before the measurements of the sex comb tooth number were conducted.

The analysis of variance was performed using the GLM and VARCOMP procedures in SAS (SAS, 1988). Composite interval mapping (Zeng, 1994), as implemented by the QTL Cartographer software (Basten

et al., 1999; <ftp://esssjp.stat.ncsu.edu/pub/qtlcart/>) was used to test the hypothesis that an interval flanked by two adjacent markers contains a QTL affecting the trait, while simultaneously controlling for the effects of markers situated outside the tested interval (for explanations, refer to Lynch & Walsh, 1997). Seventy-six unlinked cytological markers were used for the analysis with the parameters 6 (model), and 5 (window size). The conditioning markers were automatically chosen by stepwise forward-backward regression. The likelihood ratio test statistic, LR , is $-2\ln(L_0/L_1)$, where L_0/L_1 is the ratio of the likelihood under the null hypothesis (there is no QTL in the interval) to the alternative hypothesis (there is a QTL in the interval). An empirical distribution of the LR test statistics under the null hypothesis of no association between any of the intervals and trait values was obtained by randomly permuting the trait data 1000 times and calculating the maximum LR statistics across all intervals for each permutation. LR statistics from the original data that were exceeded fewer than 50 times by the maximum LR statistics from the permutation are significant at $P=0.05$ (see Doerge et al., 1997; Manly & Olson, 1999, for discussion of limitations of this analysis).

Results and discussion

The sex comb tooth number per leg scored 10.5 in Oregon R and 9.1 in 2b lines, with the difference being significant ($F_{1,78} = 31.9$; $P < 0.0001$). To identify QTLs accounting for this intraspecific variability in sex comb tooth number, we screened sex combs on each leg of 40 males from each RI line. The line component of the tooth number variance (13.4%) was significant again ($F_{97,3822} = 14.89$; $P < 0.0001$). Neither side (left vs. right leg, fixed effect), nor side by line interaction (random effect) significantly contributed to the variance.

There were two regions of the *D. melanogaster* X chromosome for which LR at each marker exceeded the threshold value 11.58 (Fig. 1). One QTL between markers 3E and 6E accounted for -0.23 teeth, and another between markers 12E and 19 A accounted for 0.34 teeth. These QTL positions should be taken with caution (Zeng, 1994). The joint effect of QTLs (0.11 teeth) explained only 8% of the difference between the parental lines in tooth number. To explain this pattern we suggest that there is a large number of small-effect sex comb QTLs segregating between the Oregon R and 2b lines, which we are not able to detect because of the limited power of our analysis.

Fig. 1 The intraspecific sex comb QTLs. Panels (a–c) represent three major *Drosophila* chromosomes (X, 2, and 3). Lines depict likelihood profiles (log likelihood ratio – LR). Horizontal lines show statistic thresholds for $P=0.05$.

In the analyses of quantitative variation for abdominal and sternopleural bristle number, segregation of a few major-effect chromosome regions frequently explained most of the variance (see Gurganus *et al.*, 1998 for a recent example and citations to earlier papers). However, the intervals in which these QTLs resided were rather large, approaching 1/20 of the genome. It is estimated that there are approximately 20 000 genes in *Caenorhabditis elegans* and, perhaps, the same in *Drosophila*. Thus, the QTL support intervals typically include 1000 genes, leaving unresolved the issue of whether there were a few factors with large effects or multiple linked factors with small effects within the interval (Lynch & Walsh, 1998).

For instance, Long *et al.* (1995) mapped a QTL to the region 89D–92E, which was responsible for 3.6 and 2.4 abdominal bristles, and 1.2 and 1.6 sternopleural bristles in males and females, respectively. Long *et al.* (1998) associated bristle number with molecular polymorphisms segregating in *Delta* and *Hairless*, candidate genes in the bristle formation pathway situated in the mapped interval. Two identified polymorphisms were specific to either abdominal or sternopleural bristles and had much smaller effects than the effects of QTL alleles. Although the QTL mapping experiment may have overestimated, and association studies underestimated, these allelic effects (Long *et al.*, 1998), the discrepancy could also be explained if there were many mutations in different genes of the region 89D–92E. The most thoroughly studied story — molecular genetic dissection of quantitative variability of *Adh* expression — points to the latter explanation. Indeed, Stam & Laurie (1996) found that many segregating genes contributed to the variability of *Adh* activity. Moreover, differences in activity between slow and fast *Adh* alleles were accounted for primarily by interactions of many nucleotide substitutions within the *Adh* locus. To infer the distribution of allelic effects for morphological quantitative traits, one clearly needs to identify genes and their polymorphisms instead of chromosomes and their regions.

QTL mapping is, thus, just the first step in inferring the genetic architecture of quantitative traits. The next step is to proceed from QTL to genetic locus. One approach is to search for candidate genes identified by mutations of large effects in the region to which the QTL maps (Mackay, 1995). The biochemical and developmental processes responsible for sex comb formation are among the best understood. Thus, one may hypothesize which genes contribute to interspecific variation in this trait. Most of these genes are in the *Polycomb* group (*Pc-G*). It has been estimated that there may be as many as 40 *Pc-G* genes (Landecker *et al.*, 1994). About 15 *Pc-G* genes have already been described (see Simon, 1995 for review), and more data are likely in the coming

years. The *Pc-G* genes are required for maintaining anterior restriction on the expression of the homeotic selector genes of the *BX-C* and *Antp-C*, and hence for the maintenance of segmental determination during embryonic development (Landecker *et al.*, 1994). Double or triple mutants of *Pc-G* genes have synergistic effects in homeotic transformations compared with single mutations (Messmer *et al.*, 1992). Two smaller groups of sex comb modifiers are represented by genes involved in the terminal stages of leg segmentation, and by genes directing sex-specific expression of somatic cells. A long list of candidate genes for which mutations might be responsible for sex comb variability in nature is available. The positions of some of these candidates match the positions of QTLs found by True *et al.* (1997) and mapped here (Table 1). Among those, mutations of candidate genes *sexcombless* and *sexlethal* seem to be the most promising candidates for future work, as we frequently observed phenotypes intermediate between males and females for the sex comb teeth. Partial rotation of the row and interrupted rows (data are not shown) might be indicative of the weak alleles of these genes.

Sex comb tooth number could, perhaps, be modified by mutations in genes in the bristle formation pathway (see Mackay, 1995, for a review) because sex comb teeth are modified bristles. True *et al.* (1997) found the largest effect QTL in the region 73A–84BD. This region also affected anal plate bristle number, clasper bristle number and the number of bristles on the 5th sternite (True *et al.*, 1997). The RI lines we employed here were previously used to map QTLs for the number of sternopleural and abdominal bristles (Gurganus *et al.*, 1998). The chromosome interval 3E–6E influencing sex combs, affected also the number of sternopleural but not abdominal bristles. In both cases, either pleiotropic effects of a single gene involved in a bristle formation pathway (or sex-specific cell fate pathway), or linkage of genes affecting different traits might be responsible. Although single mutations affecting one bristle trait frequently affect other traits as well (Lyman *et al.*, 1996), most bristle genes have effects limited to very specific patterns (Garcia-Bellido, 1979). Finer-scale genetic dissection is required to choose between these explanations.

Here, we started to dissect the genetics of intraspecific variability in sex combs to test whether standing variation in this quantitative trait serves as a substrate for selection response during speciation. The same chromosome region 12E–19A contributed to both intraspecific variability in *D. melanogaster* and interspecific differences between *D. mauritiana* and *D. simulans*. Whether or not these effects can be attributed to the same genes requires further examination.

Table 1 Candidate genes for sex comb tooth number in *Drosophila melanogaster*

QTL	Candidate gene†	Function/phenotype	Position/reference
3E–6E ^{N‡}	<i>polyhomeotic proximal</i> (<i>polyhomeotic distal</i> or <i>reduplicated-sex-combs</i>)	Leg transformation	2D2–2D2§
	<i>cramped</i>	Small extra sex combs on second tarsal segment	3C2–3C2§
	<i>Sex lethal</i>	Sex identity	6F5–6F5§
10A–19E ^T	<i>sex comb distal</i>	One to two sex comb teeth on second tarsal segment	1–30.6, approximately 9A§
12E–19A ^N	<i>enhancer of morula</i>	Reduced sex combs	1–56, approximately 13–15††
	<i>sexcombless</i>	Strongly reduced teeth number	X, position is unknown§
59C ^T	<i>Polycomblike</i>	Leg transformation	55A4–55A4¶
	<i>Distal-less</i>	Variable loss of distal leg segments	60E5–60E6¶
73A–84BD ^T	<i>brahma</i>	Dominant suppressor of <i>Pc</i> , <i>Pcl</i>	72A3–72A5¶
	<i>kohtalo</i>	Suppressor of <i>Antp</i> , <i>Msc</i> , <i>Mrt</i> , <i>Pc</i> , <i>Pcl</i>	76B1–76D5¶
	<i>verthandi</i>	Suppressor of <i>Pc</i>	3–46 (76B–77F)¶
	<i>Su(z)12</i>	Additional sex comb teeth on the second and third legs	76E1–77B9¶
	<i>Polycomb</i>	Leg transformation	78D1¶
	<i>proboscipedia</i>	Transformation of leg segments	84A5–84A5¶
	<i>Sex Comb Reduced</i>	Partial leg transformation, sex comb size reduction	84A–B¶
	<i>Antennapedia</i> (<i>Sex comb</i> , <i>extra proboscipedia</i>)	Leg transformation	84B1¶
	<i>Thickened arista</i>	Extreme reduction in the teeth number	84C1–84C2¶
	<i>doublesex</i>	Sex comb teeth are intermediate in number, orientation, and position between males and females	84E1¶
	<i>Sex comb on midleg</i>	Sex comb on mesothoracic legs	85E1–10¶

†Minute mutations usually affecting sex comb teeth number are not listed.

‡T and N mark candidate genes mutations in which might correspond to QTLs mapped by True *et al.* (1997), or by those mapped here. References: §Lindsley & Zimm (1992); ¶Saget *et al.* (1998); ††Reed (1992).

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