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Genetics of female mate discrimination of heterospecific males in *Nasonia* (Hymenoptera, Pteromalidae)

BEREND-JAN VELTHUIS, WENCAI YANG, TIM VAN OPIJNEN & JOHN H. WERREN

Department of Biology, University of Rochester

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Despite its importance to mating system evolution, sexual selection and reproductive isolation, little is known about the genetic basis of female mate choice. We studied the behavioural and genetic basis of interspecific mate discrimination in *Nasonia* wasps, using two strains of *N. longicornis* (LCa and LId) that differ in female acceptance of males from the sibling species *N. vitripennis*. Both strains showed low acceptance ($\leq 2\%$) of *N. vitripennis* males in the first courtship, but acceptance by LId females subsequently increased (11% by the second and 15% by the third courtship), whereas acceptance by LCa females remained low ($\leq 2\%$). After 2 h of exposure to a *N. vitripennis* male, 59% of LId females mated compared with only 8% of LCa females. Interstrain cross F1 females showed significantly higher acceptance of *N. vitripennis* males than either parental strain in the first courtship (15–29%) and after 2 h (79–82%). A genetic analysis of acceptance at 2 h revealed three major quantitative trait loci (QTL). For each major QTL, the allele that increased female mate acceptance was dominant. The QTL with the largest effect accounted for half the strain difference, and was confirmed by additional crosses. This QTL was involved in the females' continued reluctance to mate with *N. vitripennis* males during multiple courtships. Results are discussed in terms of the behavioural and genetic architecture of female mate discrimination and possible implications to the evolution of courtship, mating systems and reproductive isolation.

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Unravelling the genetic and phenotypic structure of behaviours is key to understanding the evolutionary mechanisms of behavioural change, its causes and consequences (Lewontin 1974; Boake 1994; Foster & Endler 1999). The genetic and behavioural structure of female mate discrimination is of particular interest because of its importance in several fundamental processes in behavioural ecology, including the evolution of courtship and mating systems (Thornhill & Alcock 1983; Choe & Crespi 1997), the role of female preferences in sexual selection (Blum & Blum 1979; Bradbury & Andersson 1987; Andersson 1994) and the evolution of reproductive isolation (e.g. Hollocher et al. 1997; Tregenza & Butlin 1999; Boake 2000; Ptacek 2000). For example, female mate

discrimination is considered a driving force in sexual selection and lies at the heart of processes such as runaway (Lande 1981; Kirkpatrick 1982; Pomiankowski et al. 1991), 'good genes' (Zahavi 1975; Iwasa et al. 1991) and sensory bias (West-Eberhard 1984; Ryan 1985). The genetic structure of female preference and male traits can influence the outcome of sexual selection and reinforcement, as illustrated in theoretical models of these processes (O'Donald 1979; Iwasa et al. 1991; Liou & Price 1994; Kelly & Noor 1996; Kirkpatrick & Servedio 1999; Takimoto et al. 2000). Similarly, recent models indicate that the genetic and phenotypic bases of mate preferences and associated traits play a pivotal role in the onset of population divergence (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Takimoto 2002).

Despite its importance, little is known about the genetic basis of female mate discrimination. Several researchers have emphasized the paucity of genetic data on mate preferences and the need for more (Ritchie 1992a; Bakker & Pomiankowski 1995; Kirkpatrick & Ravnigné 2002; Shaw & Parsons 2002). Currently there are only a few studies of relevance to the genetic architecture of mate discrimination, most of which have been conducted in *Drosophila* species (e.g. Heisler 1984; Coyne 1989; Scott 1994; Boake

Correspondence and present address: B.-J. Velthuis, Department of Biology, University of Rochester, Hutchison Hall 226, Rochester, NY 14627, U.S.A. (email: velth@mail.rochester.edu). W. Yang, is now at the Department of Horticulture and Crop Science, The Ohio State University & OARDC, 1680 Madison Avenue, Wooster, OH 44691, U.S.A. T. van Opijnen, is now at the Department of Human Retrovirology, University of Amsterdam, Academic Medical Centre K3-120, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

et al. 1997; Hollocher et al. 1997; Carracedo et al. 1998; Hollocher 1998; Doi et al. 2001; Noor et al. 2001). These studies have detected major effect genes for female preference in some cases, and a highly polygenic basis in others. Studies of female preference using other insects such as crickets (Ritchie 1992b; Shaw 2000) and stalk-eyed flies (Wilkinson & Reillo 1994) have found evidence for a highly polygenic architecture, whereas major effect genes were found to be involved in mate discrimination in, for example, the European corn borer *Ostrina nubilalis* (Klun et al. 1973; Löfstedt et al. 1989; Cosse et al. 1995; Zhu et al. 1996). Finally, many behavioural genetic studies have been conducted in *D. melanogaster* in which mutations were evaluated for their (often pleiotropic) effects on female mate discrimination, male courtship and mating behaviour (reviews in: Hall 1994; Yamamoto et al. 1997).

Here we investigate the behavioural and genetic basis of female mate acceptance of heterospecific males in the parasitic wasp genus *Nasonia*. The three closely related species *Nasonia giraulti*, *N. longicornis* and *N. vitripennis* (Darling & Werren 1990) differ in various aspects of male courtship behaviour (van den Assem & Werren 1994; Beukeboom & van den Assem 2001), with *N. longicornis* males intermediate between the other two species for some, but not all traits. The genus *Nasonia* offers several advantages for a genetic analysis of female mate discrimination. Availability of natural variation in female mate discrimination (Bordenstein et al. 2000) adds to that of male courtship traits (van den Assem & Werren 1994). Genetic analysis is made easier in these wasps because of their haplodiploid sex determination. *Nasonia* males develop from unfertilized eggs and are thus haploid. Male haploidy allows the easy production of large numbers of genetically nearly identical ('clonal') females: haploid males produce genetically identical sperm (except for de novo mutations). Therefore, when males of known genotype are crossed to females of highly inbred lines, hundreds of 'clonal' daughters are produced. These genetically identical females of known genotype can then be used to accurately assess their mate discrimination phenotype. Finally, molecular and visible markers are available in *Nasonia* to assist in genetic studies of behavioural and other traits (Gadau et al. 1999, 2002; Weston et al. 1999).

The basic courtship behaviour of *Nasonia* has been described previously (Barrass 1960; van den Assem & Werren 1994) and is as follows. The male orients towards a nearby female, chases her, and mounts by climbing onto her back. The female responds by stopping her movement and allowing the male to position himself with his head above the female's antennae. He then commences a stereotypic and cyclic courtship display. If the female signals receptivity, the pair will copulate. Otherwise, the male eventually dismounts. The male may remount the female later, and persistence of males can result in several courtships during the first few minutes after the initial encounter. A more detailed description can be found in van den Assem & Werren (1994).

A recent study (Bordenstein et al. 2000) has shown considerable variation in *N. longicornis* for female mate acceptance of *N. vitripennis* males, ranging from very low

(under 5%) to high (70%), when presented with a male for 2 h. To investigate the genetic and behavioural basis of *Nasonia* mate discrimination, we selected two *N. longicornis* strains with a large difference in female acceptance of *N. vitripennis* males. We conducted a behavioural and genetic analysis of this difference and discuss our results in relation to theories of female mate preference, sexual selection and reinforcement.

METHODS

Strains and Recombinant Inbred Lines

Three inbred isofemale strains were used in the experiments. *Nasonia longicornis* strain LCa (i.e. RNLCa 9304) is a line derived from a single female collected in California, while LId (RNLI dB 418A) is derived from a single female collected in Idaho. The *N. vitripennis* strain RNVXIdB 431E, also from Idaho, was chosen for the heterospecific test male. These strains were previously used for a study of sexual isolation in *Nasonia*: LCa was found to have the lowest acceptance of *N. vitripennis* males (3% mating), and LId a consistent high acceptance (57% mating), in a 2-h mating assay (Bordenstein et al. 2000). We do not know whether these strains represent typical mate discrimination patterns in their respective geographical regions.

To validate (or not) the findings of our QTL analysis presented below, we constructed a set of recombinant inbred lines (RILs) from an initial cross between the two *N. longicornis* strains. The RILs harbour chromosomal regions from the LId strain in an otherwise LCa maternal genetic background. We constructed our recombinant inbred lines in the LCa genetic background because differences in relevant mate acceptance behaviours prove to be better resolved here than in the alternate (LId) genetic background. Initial backcrosses of LId to LCa produced several 'sibships' of genetically near-identical females (see Fig. 1). We selected some of these sibships for the construction of RILs based on both their female mate acceptance behaviour and on the genotype of their respective fathers (see Fig. 1 and below). Selected genotypes or 'lines' were made homozygous by mating a sibship virgin female to one of her sons and mating this son to one of his sisters and subsequently to one of his daughters (if possible). We conducted the initial mother-son crosses by setting a virgin *Nasonia* female on two hosts, and then keeping her alive at 4°C for 12–14 days while her (male) progeny developed at 25°C. Each mother was then mated to one of her sons. We used a similar procedure for the subsequent father-daughter cross a generation later, but with a slightly lower success (if a male died prematurely, we conducted an additional mother-son cross in the next generation). Haploidy of males ensures that parts of the genome quickly become homozygous in the female progeny resulting from these cross-generation familial matings. Subsequently, 7–10 generations of brother-sister mating and single-female transfer (i.e. each subsequent generation was derived from just one sibmated female) resulted in a continual reduction of the size of heterozygous regions. After this

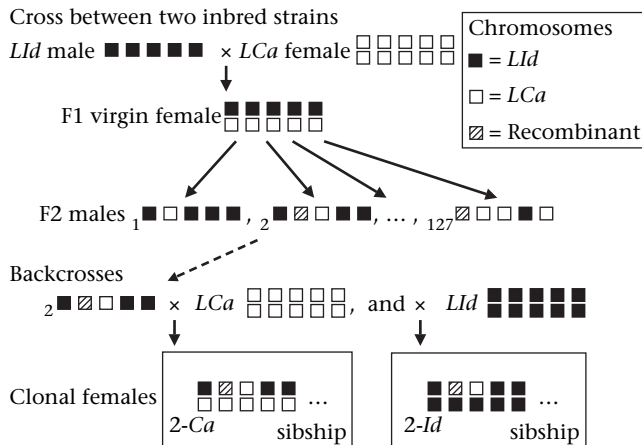


Figure 1. Advantages of haplodiploid genetics: ‘clonal’ backcross sibships. *Nasonia* males are haploid (five chromosomes), females are diploid. A cross between the inbred strains *Lld* and *LCa* gives genetically identical F1 females. Virgin F1 females produce male offspring only. An F2 male is a unique haploid genetic mixture of the two strains. His sperm has a near-singular genotype (no meiosis). Reciprocal backcrosses produce large numbers of ‘clonal’ females (i.e. sibships). Replicate testing of such females allows for accurate quantification of phenotypic differences. Comparisons across sibships with the same type mother point to differences in mate acceptance caused by genetic differences in their respective F2 fathers, and vice versa.

procedure, less than 1% of the genome was estimated to not yet be homozygous. The recombinant inbred lines so generated differ in both the location and size of chromosomal regions that are homozygous for *Lld* alleles in an otherwise homozygous *LCa* background.

Experimental Designs

We used two assays to measure differences between *LCa* and *Lld* in female mate discrimination with respect to *N. vitripennis* males. In the first, we paired single *N. vitripennis* males and *N. longicornis* females for 2 h. Matings were assayed indirectly by the production of female offspring (due to haplodiploidy, mated females produce both daughters and sons, whereas unmated virgin females produce sons only). Previous studies have shown that this is a reliable index of mating in both intraspecific and interspecific crosses (Bordenstein et al. 2000). After the 2-h exposure, females were provided with blowfly hosts for oviposition, and the sex of emerging offspring was scored.

In the second assay, we observed courtship behaviour directly. Single males and females were paired for 15 min in no-choice mating trials, in which relevant behaviours (e.g. number of courtships, receptivity of female and successful copulations) were recorded. Both assays employed naïve virgin females and males of fixed age (1–3 days postemergence), as age has previously been shown to influence mating propensity. All experiments were conducted using single male, single female mating pairs. First, *LCa* and *Lld* females, and F1 females from reciprocal crosses between the two strains were tested for

their acceptance of a *N. vitripennis* male. Later, ‘clonal’ backcross females (see below) and females from *LCa*/*Lld* recombinant inbred lines were similarly tested. Female acceptances of both *LCa* and *Lld* males served as controls. All females and males were used only once.

Phenotyping of ‘clonal’ Recombinant Genotype Females

An important advantage of *Nasonia*'s haplodiploid genetic architecture is the ability to easily generate large numbers of genetically near-identical females with recombinant genotypes. This allows us to reduce the variance associated with behavioural phenotypes by replicate testing of each genotype. The procedure is as follows (see Fig. 1). F1 females from a cross between an *Lld* male and an *LCa* female are set on hosts as virgins, to produce F2 males. The sons of F1 females are haploid recombinant genotypes between the two strains. Because *Nasonia* males are haploid, all the sperms produced by a male are genetically identical. Each F2 male is backcrossed to females from both parental strains. From the backcross to females of either strain, a set of daughters is produced with near-identical genotypes, composed of half of the father's haploid genotype and half of the mother's genotype. Unlike the case in diploids, *Nasonia* backcross females so generated are ‘clonal’ (i.e. sibships). We determined the genotype of a sibship from the genotype of their respective F2 father and that of either *LCa* or *Lld* (see below, and Fig. 1). For each ‘clonal’ backcross sibship, we tested females for their acceptance of *N. vitripennis* males using the 2-h assay. Controls of *LCa* and *Lld* females tested with a *N. vitripennis* male were run alongside each batch of ‘clonal’ sibships.

Phenotyping of Recombinant Inbred Lines

Females from 36 recombinant inbred lines (RILs) were tested for their mate acceptance of a *N. vitripennis* male, using the 2-h assay. The first set of 18 RILs all harboured fairly large chromosomal regions that were homozygous *Lld*. These lines were chosen because they might readily reveal the (high mate acceptance) phenotype of an *Lld* chromosomal region in an otherwise discriminating (*LCa*) genetic background. The second set of 18 RILs, with only small *Lld* regions, was also tested. In these RILs, effects of individual QTL can be measured more accurately in the absence of cosegregating QTL. Both sets of RILs were tested for their female acceptance of *N. vitripennis* males using the 2-h assay, using *LCa* and *Lld* females for controls. Selected RILs were also tested using the 15-min observation assay. The genotype of RILs was determined using a subset of molecular markers used to screen the F2 males.

AFLP Genotyping

We screened parental strains *LCa* and *Lld*, F2 males and recombinant inbred lines for molecular markers using amplified fragment length polymorphism (AFLP) techniques (Vos et al. 1995; Rouppe van der Voort et al. 1997;

Mueller & Wolfenbarger 1999). AFLP markers are dominant, but when genotyping haploid males, both presence and absence of markers are informative. The AFLP kits were obtained from Applied Biosystems (Perkin-Elmer, Boston, Massachusetts, U.S.A.). Combinations of *EcoR* I and *Mse* I primers from the AFLP kit were used; specifics are available from the authors upon request. AFLP gels were run on an ABI 377 genetic analyser, using three different fluorescent dyes (FAM, JOE and NED) and either GS GenoTYPE 500 ROX (Perkin-Elmer) or Gibco 50-500 ROX (Invitrogen, Carlsbad, California, U.S.A.) for the internal lane size standard.

A total of 80 reliable AFLP markers were found between LCa and LId, 59 of which were chosen to score the 127 F2 males; 32 LCa-specific markers, and 27 LId-specific markers. Only four and three, respectively, of these markers showed a segregation ratio significantly different from 1:1 in the F2 males (chi-square tests: $P < 0.01$).

Data from the gels were extracted using Genescan, version 3.1 (Applied Biosystems 1998), and genotyping was performed by visual inspection of gel images constructed using the program Genographer, version 1.6.0 (Benham 2001). Manual genotyping proved a necessity, because bands on an AFLP gel can differ greatly in intensity and may vary among gels due to slight differences in PCR and gel running conditions. Reamplified samples were run when the absence of a marker band was in doubt, based on the intensity of nearby bands on the gel image. Absence of a marker band may be taken to indicate actual absence of the respective DNA fragment (and thus the alternate genotype for that marker locus), unless nearby nonpolymorphic bands are also absent or too faint. This later case would suggest reamplification to be necessary, or the data to be scored as missing instead.

Intraspecific Linkage Map(s)

We used a population of 127 F2 males, scored for 59 AFLP markers, to generate an intraspecific LCa/LId linkage map. The programs Mapmaker, version 1.2 (Lander & Green 1987; Lander et al. 1987), PGRI (Liu 1998) and JoinMap (van Ooijen & Voorrips 2001) were all used to determine linkage and evaluate order of markers on each of the linkage groups. With a mapping population of this size, linkage mapping softwares tend to give different maps (due to different algorithms and criteria for linkage; see e.g. Olsen & Boehnke 1990), which then need to be evaluated to arrive at a consensus map. Confidence in the linkage map was determined by jackknife and bootstrap procedures implemented in PGRI (Liu 1998); for example, dropping a correctly placed marker tends to have only local effects on marker order, given sufficient density of markers on that linkage group, whereas dropping an incorrectly placed marker may result in profound rearrangements of (sets of) markers. Confidence in marker order is highly relevant because QTL analyses based on interval mapping implicitly assume that the underlying marker order is correct, although not always with proper justification (Stringham & Boehnke 2001). The 'percentage of correct order' (PCO) value represents the number of

times a given marker order was found in 100 bootstrap samples (Liu 1998), and provides a measure of confidence in our linkage groups.

We consistently found seven linkage groups: LG 1, 2a, 2b, 3, 4, 5 and 6. Two of them (LG 2a and 2b) formed a single linkage group when less stringent linkage criteria were used, but ordering of markers was much improved by treating them as two loosely linked linkage groups. The number of markers, the total length in centi-Morgans, and the bootstrap support value (PCO) for each of the major linkage groups were as follows: LG 1, 9 markers, 50 cM, PCO = 35%; LG 2a, 6 markers, 32 cM, PCO = 75%; LG 2b, 7 markers, 37 cM, PCO = 75%; LG 3, 9 markers, 61 cM, PCO = 79%; LG 4, 6 markers, 23 cM, PCO = 87%; and LG 5, 5 markers, 6 cM, PCO = 58%. Five markers were excluded from the linkage map due to their adverse effect on marker order, and a single marker could not be assigned to any linkage group.

We obtained additional information on marker order by genotyping 22 RILs, and by using a separate F2 mapping population of LCa crossed with several eye-colour mutant strains of *N. vitripennis* (data not shown). Combining data from the three mapping populations sufficiently improved our intraspecific linkage map for the purpose of interval mapping of QTL. We also used parsimony (e.g. number of double crossovers between markers should not exceed single crossover events) and common sense (e.g. 'allelic' markers should map to the same or similar position) to produce a linkage map that incorporated 57 of our 59 AFLP markers (Fig. 2).

The six linkage groups in the resulting linkage map compare favourably to the five chromosomes known to occur in *N. longicornis* by cytological analysis (Gokhman & Westendorf 2000), but also indicate further work is still needed to fully resolve the map. By using visible markers that identify particular chromosomes in *N. vitripennis*, we tentatively assigned linkage group 3 (STDRTet) of the LCa/LId linkage map to be homologous to chromosome 'I' in *N. vitripennis* (cf. Fig. 2 and Gadau et al. 1999). The remaining linkage groups could not be unambiguously assigned to chromosomes.

QTL Analysis

We sought to identify genetic regions affecting female acceptance of a *N. vitripennis* male. Our QTL analysis was based on the 2-h assay, using the two populations of 'clonal' backcross sibships previously described; 119 sibships with the LCa maternal genetic background, and 83 sibships with the LId maternal genetic background. The two populations were screened for QTL separately, but the fact that each of 83 F2 fathers were shared between a pair of sibships, one from each population, allowed us to use results across both genetic backgrounds. This feature, plus the ability to replicate genotypes for phenotypic quantification of sibship female mate acceptance behaviour, greatly increased our resolution to detect QTL, despite the relatively small size of our F2 mapping population.

Identification of putative QTL involved a conservative, three-step procedure. First, a single marker QTL analysis

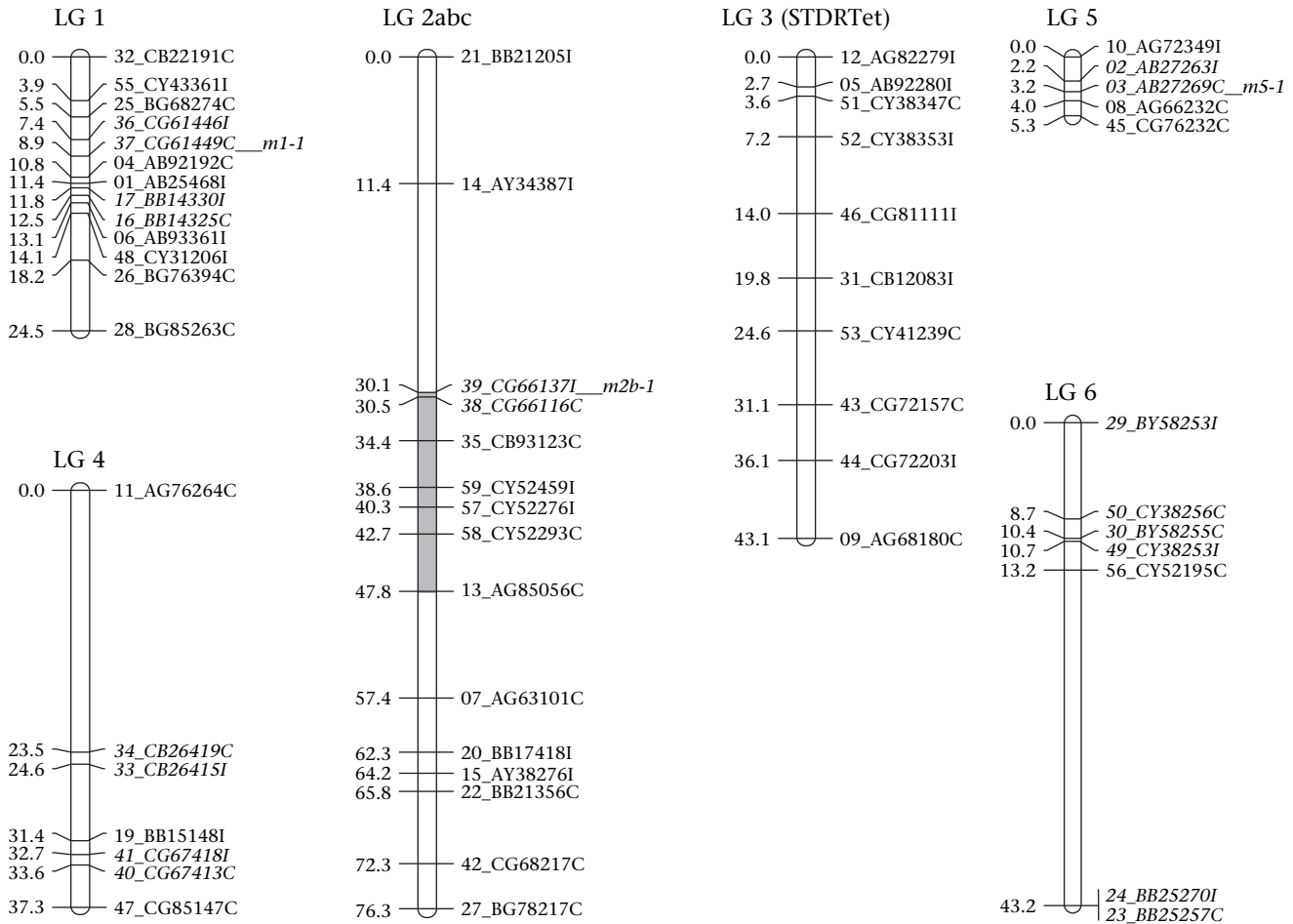


Figure 2. Intraspecific AFLP linkage map for *N. longicornis* (LCa/Lld) based on 127 F2 males. Additional linkage information from 22 LCa/Lld recombinant inbred lines and 48 hybrid *N. longicornis*/*N. vitripennis* F2 males was used to improve the order of markers in linkage groups 1, 2abc, 4 and 5. The shaded part of LG 2abc represents LG 2b from the preliminary linkage map. Specific markers linked to major QTL are indicated with m1-1, m2b-1 and m5-1, respectively. Positions of markers are shown on the left (in cM); we have used a Kosambi mapping function. The final map was drawn using Mapchart 2.1 (Voorrips 2002).

was conducted for each genetic background separately. This identified markers linked to major QTL. Second, we conducted a ‘whole linkage group’ analysis (i.e. using only individuals for which one or more linkage groups were nonrecombinant) to evaluate associations of linkage group genotype with the sibship’s female mate discrimination behaviour and to analyse genetic interactions between linkage groups that affect the phenotype. This step allowed us to differentiate putative QTL from so called ‘ghost QTL’ (i.e. false positives that merely arise as a consequence of cosegregation of a major QTL on another linkage group). Finally, as a last step, we used the program MapQTL (van Ooijen et al. 2002) to map the major QTL identified in the first two steps more accurately, and to measure their effects. Here we used the MQM procedure (Jansen 1993; Jansen & Stam 1994) and interval mapping (Lander & Botstein 1989) based on the improved intraspecific linkage map (see above). Introgression of Lld-specific AFLP markers into the LCa genetic background of RILs then enabled us to ultimately confirm the location of a major QTL and study its effect(s) in a more detailed behavioural characterization.

Statistics

Phenotypic data presented in this paper measure the proportion of females accepting a particular type of male as a mate. We used sample size (50–80 females per strain or sibship) to calculate a 95% confidence interval around such proportions. Statistical significance of a difference in proportion mate acceptance was determined using a two-tailed Z test directly on proportions (Daniel 1999, page 252), or when appropriate, a two-tailed *t* test, assuming unequal variances (Daniel 1999, page 232), on arcsine square-root transformed proportions (Sokal & Rohlf 1998, page 419).

For the QTL analyses on ‘clonal’ sibships, we determined the effect of genotype at markers linked to QTL using a Wilcoxon–Mann–Whitney test (Siegel & Castellan 1989) on class medians. Classes of sibships with the same genotype at a particular marker or set of markers were formed. The proportion of mate acceptances of these sibships was arcsine square-root transformed, and the within-class median values were calculated and compared. For the single marker QTL analyses and the ‘whole linkage

group' analyses, we applied a Bonferroni correction (Bland & Altman 1995; Lander & Botstein 1989; Bender & Lange 2001). For the MQM mapping of major QTL, we used a permutation test (Churchill & Doerge 1994), implemented in the program MapQTL, to determine linkage group and genome-wide (i.e. experiment-wise) LOD thresholds; that is, a threshold value above which the 'likelihood of odds' indicates that the presence of a QTL explains the phenotypic data better than does its absence (Lander & Botstein 1989; Lynch & Walsh 1998). The software Epistat (Chase et al. 1997) was used to search for epistatic interactions between QTL. This program uses log-likelihood ratios to compare the likelihood of explaining QTL effects by null, additive, or epistatic models.

RESULTS

Basis of Heterospecific Male Acceptance Differences

Bordenstein et al. (2000) found considerable variation among field-collected *N. longicornis* strains in female acceptance of males from the related species *N. vitripennis* when tested in a 2-h mating assay. We first repeated this 2-h assay and confirmed the LCa and LId strain difference with respect to acceptance of *N. vitripennis* males of the particular strain (RNvXIDB 431E) chosen for our current study. Our results are consistent with the earlier study performed with several different *N. vitripennis* strain males (Bordenstein et al. 2000). An average of 8.3% of LCa females versus 59.1% of LId females accepted the *N. vitripennis* male during 2 h of exposure. These results are based on large sample sizes (LCa: 18 replicated experiments with a mean of 74 females per replicate; LId: 17 replicates with a mean of 66 females per replicate), and the difference was highly significant (Wilcoxon–Mann–Whitney test: $W = 182.5$, $N_1 = 18$, $N_2 = 17$, $P < 0.0001$). No differences were detected between LCa and LId females with respect to their acceptance of conspecific males from either *N. longicornis* strain (all close to a 100%). We also examined acceptance of males by F1 females in reciprocal LCa–LId interstrain crosses. F1 females showed high acceptance levels of *N. vitripennis* males, significantly higher than LCa (t test: $t_{1471} = 231.8$, $N_1 = 141$, $N_2 = 1334$, $P < 0.0001$) and LId females (t test: $t_{1251} = 67.5$, $N_1 = 141$, $N_2 = 1114$, $P < 0.0001$). Conspecific males were accepted by F1 females at high levels (~95%), similar to levels of both parental strains.

Direct observations of courtship were not conducted during the 2 h interval. We therefore undertook a more detailed behavioural analysis to investigate the phenotypic basis of this acceptance difference, using both parental strains and F1 interstrain cross females. Virgin females were individually paired with either *N. longicornis* or *N. vitripennis* males and observed for 15 min.

Observations of LCa and LId females paired with *N. vitripennis* males revealed that both females tended to reject *N. vitripennis* males in the first encounter (Fig. 3, Table 1). LCa females and LId females mated with *N. vitripennis* males 1% and 2% of the time in the first

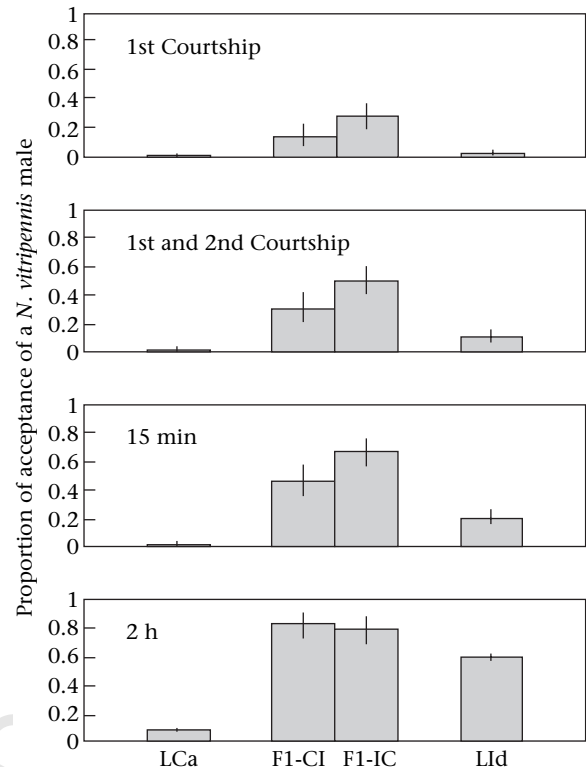


Figure 3. Acceptance of a *N. vitripennis* male as a mate by females of the two *N. longicornis* strains LCa and LId and by both types of reciprocal cross F1 females (F1-CI from crossing an LCa male \times LId female, and vice versa for F1-IC). Proportions of mate acceptance are shown with 95% confidence limits for the first courtship, cumulative over the first and second courtship, and at the end of the 15-min behavioural observation assay, as well as from a separate 2-h mating assay. See text and Table 1 for sample sizes, statistical tests and discussion of differences.

courtship, respectively. This contrasts with 80–86% acceptance when either female was paired with conspecific males of either *N. longicornis* strain (Table 1). However, in subsequent courting, LId female acceptance of *N. vitripennis* males increased significantly (11% acceptance by the second courtship; Z test: $Z = 3.52$, $N_1 = N_2 = 212$, $P = 0.0002$; 15% by the third courtship; Z test: $Z = 4.87$, $N_1 = N_2 = 212$, $P < 0.0001$), whereas LCa females remained reluctant to mate with *N. vitripennis* (1% at both second and third courtship). LId females changed their mate acceptance behaviour rapidly; within the first 3 min after their initial encounter with a *N. vitripennis* male, the difference between LCa (1%) and LId (8%) was already significant (Z test: $Z = 3.32$, $N_1 = 207$, $N_2 = 212$, $P = 0.0005$). LId females in this assay took approximately 3 min to encounter and accept a conspecific male, whereas LCa female acceptance was still only 2% compared with 20% for LId by the end of the 15-min observation period.

Mate acceptances of *N. vitripennis* males by F1 interstrain females are also presented in Fig. 3. Whereas both parental strains showed low acceptance of *N. vitripennis* males in the first courtship, F1 females of both reciprocal crosses (F1-IC from an LId male \times LCa female, and F1-CI from the reciprocal cross) showed significantly higher

Table 1. Behaviour assay: female mate acceptance of conspecific and heterospecific males

Female	3 min		15 min	First courtship		N	
	Male		V%	Male			
	L%	V%		L%	V%	L	V
V	15	71	96	10	85	21	171
LCa	63	1	2	80	1	114	207
	$P < 0.001$		$P < 0.001$				
Lld	81	8	20	86	2	111	212
F1-CI	94	15	47	100	15	17	75
			$P = 0.006$		$P = 0.016$		
F1-IC	92	14	66	100	29	37	91

Percentage of female mate acceptances of *N. longicornis* (L) and *N. vitripennis* (V) males 3 and 15 min after placing the male and female together, and for the first courtship only. *N* = number of pairs observed. Significant differences in percentages of acceptance of a *N. vitripennis* male for the comparison of the two parental strains as well as for that of reciprocal F1 females are shown (two-tailed Z test); see text for other comparisons. F1 females from reciprocal crosses between the LCa and Lld strains are indicated as F1-CI and F1-IC.

acceptance than either parental strain (F1-CI: 15% versus Lld: 2%; *t* test: $t_{283} = 25.2$, $N_1 = 75$, $N_2 = 212$, $P < 0.0001$; F1-IC: 29% versus Lld: 2%; *t* test: $t_{299} = 68.5$, $N_1 = 91$, $N_2 = 212$, $P < 0.0001$; LCa: 1% ≤ Lld: 2%; see also Table 1). This result was surprising, because the higher first courtship acceptance 'emerged' from two strains with extremely low acceptances. F1 females also showed increased acceptance of conspecific *N. longicornis* males at the first courtship (Table 1: pooled F1: 100% versus LCa: 80%; *t* test: $t_{164} = 45.4$, $N_1 = 54$, $N_2 = 114$, $P < 0.0001$; pooled F1: 100% versus Lld: 86%; *t* test: $t_{161} = 36.6$, $N_1 = 54$, $N_2 = 111$, $P < 0.0001$), suggestive perhaps of a single threshold for mate acceptance in general. By 15 min and 2 h, both F1 females and Lld females showed elevated acceptance of *N. vitripennis* males, although F1 females' acceptance remained significantly higher than that of Lld females; (15 min: F1-CI: 47% versus Lld: 20%; *t* test: $t_{273} = 40.3$, $N_1 = 75$, $N_2 = 212$, $P < 0.0001$); F1-IC: 66% versus Lld: 20%; *t* test: $t_{298} = 82.3$, $N_1 = 91$, $N_2 = 212$, $P < 0.0001$; 2 h F1-CI: 82% versus Lld 59%; *t* test: $t_{175} = 38.7$, $N_1 = 67$, $N_2 = 1114$, $P < 0.0001$; F1-IC: 79% versus Lld: 59%; *t* test: $t_{1181} = 29.4$, $N_1 = 71$, $N_2 = 1114$, $P < 0.0001$).

Furthermore, in the 15-min assay, F1 females differed in acceptance based on the cross direction (Fig. 3, Table 1). F1 females with an Lld father (F1-IC) showed a significantly higher acceptance of *N. vitripennis* males than did the F1 females with an LCa father (F1-CI), in the first courtship (29% versus 15%; Z test: $Z = -2.14$, $N_1 = 91$, $N_2 = 75$, $P = 0.016$), and after 15 min (66% versus 47%; Z test: $Z = -2.50$, $N_1 = 91$, $N_2 = 75$, $P = 0.006$).

In summary, our results show that both *N. longicornis* strains strongly rejected *N. vitripennis* males in the first courtship, but that Lld strain females showed increased acceptance of *N. vitripennis* in second and later courtships, whereas LCa females continued to reject. F1 females showed a markedly higher acceptance of *N. vitripennis*

males than did females of either parental strain, and this was already apparent in the biologically most relevant first courtship.

Genetic Analysis of Female Mate Acceptance Differences

We tested 50–80 females from each of 119 sibships with the paternal chromosomes in the LCa genetic background and from each of 83 sibships with the paternal chromosomes in the Lld genetic background for mate acceptance of a *N. vitripennis* male in the 2-h assay. Nine sibships were replicated to determine the degree of consistency in their mate acceptance. Across these sibships, the average ± SE difference in the percentage of acceptance between the two replicates was $7.0 \pm 2.1\%$, $N = 9$, indicating a good replicability of each sibship's phenotype.

Segregation of Lld alleles in the LCa genetic background yielded a distribution of phenotypes (data not shown) spanning the entire range (0–100%) of female mate acceptance, whereas only 7% of the sibships in the Lld maternal genetic background showed a level of mate acceptance below that of Lld (59%). Comparison of the distributions of mate acceptance across sibships in the two genetic backgrounds confirmed the result from the F1 analysis that acceptance alleles tended to be dominant relative to discrimination alleles. A comparison of sibships resulting from backcrossing an F2 male to Lld and from backcrossing the same F2 male to LCa, revealed that most haploid recombinant genotypes in an Lld maternal genetic background had a higher acceptance of *N. vitripennis* males than those in an LCa background. Of 83 such pairs, 73% showed higher acceptance when in an Lld background, with an average acceptance of 79%, versus 60% for an LCa background (Wilcoxon signed-ranks test: $Z = 4.227$, $N = 83$, $P < 0.00003$).

Phenotypes of some sibships were significantly more extreme than either parental strain. These 'transgressive' phenotypes were observed in both genetic backgrounds: 19% of sibships in the LCa background and 43% of sibships in the Lld background were significantly ($P < 0.025$) higher in mate acceptance than Lld, based on 95% confidence limits. In the LCa background, 11% of sibships were well below the LCa level of 8.3% acceptance of a *N. vitripennis* male (data not shown). This suggests that not all LCa alleles are recessive, and that not all LCa alleles decrease female mate acceptance of *N. vitripennis* males. The QTL analysis (see below) confirms this interpretation.

QTL Analysis of Female Mate Discrimination

Here we summarize the single marker and 'whole linkage group' analysis and then describe the MQM QTL analysis. The single marker analysis showed highly significant effects (*t* tests: $P < 0.0059$, after Bonferroni adjustment) within the LCa genetic background for three markers, two on linkage group 1 (designated m1-1 and m1-2) and one on linkage group 2 (designated m2b-1).

Substitution of an Lld allele for an LCa allele at the major QTL increased the mean proportion acceptance of *N. vitripennis* males by 23% for m1-1 (42% to 65%), 28% for m1-2 (39% to 67%) and 34% for m2b-1 (33% to 67%). A statistically nonsignificant increase in mate acceptance was found for an LCa allele on linkage group 5 (m5-1); its biological significance arises from the fact that in this case an allele from the more discriminating strain (LCa) actually increased acceptance of a *N. vitripennis* male. Its relevance becomes more apparent when examining QTL in the Lld background. In the Lld genetic background, the only significant QTL was m5-1 on LG 5 (t test: $t = -5.379$, $P < 0.0001$, Bonferroni adjusted $P < 0.0059$). Here, the LCa allele increased the mean proportion acceptance of a *N. vitripennis* male by 13% (56% to 69%). Therefore, for all these QTL, the acceptance alleles appeared to be dominant relative to discrimination alleles.

The next analysis used only nonrecombined linkage groups (e.g. all markers on linkage group 1 had LCa alleles) to further evaluate effects. This analysis confirmed the presence of major QTL on the three linkage groups described above. We partitioned the sibship data sets, using unrecombined linkage groups harbouring major QTL as cofactors, to differentiate 'real' effects from 'pseudo' effects that arise from cosegregating QTL, and to investigate possible interactions between linkage groups. We found that LG 1 and LG 5 had a significant effect in both genetic backgrounds, whereas LG 2b had an effect only in the LCa background (see Table 2). The program Epistat (Chase et al. 1997) was used to determine significance of nonadditive interactions between markers linked to major QTL. No such nonadditive gene-by-gene interactions could be detected between any pair of markers on LG 1 and LG 2b, in either genetic background. However, in the LCa genetic background, a significant

conditional epistatic interaction (Monte Carlo: $P < 0.01$) was found between major QTL m5-1 and m2b-1; when m2b-1 was homozygous LCa/LCa, a significant effect at QTL m5-1 was detected (Wilcoxon–Mann–Whitney test: $W = 219.0$, $N_1 = 16$, $N_2 = 24$, $P < 0.034$, after Bonferroni correction). In the Lld genetic background, a significant conditional epistatic interaction (Monte Carlo: $P < 0.03$) was detected between markers m5-1 and m1-1. A single LCa allele at either locus resulted in a significant increase in acceptance of *N. vitripennis* males (Wilcoxon–Mann–Whitney tests: LG 1: $W = 376.5$, $N_1 = 17$, $N_2 = 16$, $P < 0.0036$; LG 5: $W = 360.0$, $N_1 = N_2 = 16$, $P < 0.0008$). But, when both linkage groups were heterozygous, no significant additional increase was detected (Table 2).

We further analysed major QTL by the MQM cofactor method and interval mapping procedure of the program MapQTL, using our consensus linkage map (Fig. 2). This analysis confirmed our basic findings and located more accurately the positions of major QTL within each linkage group (Fig. 4). The key findings from the MQM analysis were as follows. Only three, relatively small genetic regions harbouring major QTL were detected, on linkage groups 1, 2 and 5, respectively. Each of these regions spanned about 5 cM in the current linkage map of 230 cM total length (i.e. ~2% each, or 6% total). No major QTL could be detected on linkage groups 3, 4 and 6. Furthermore, large regions of LG 1 and 2 appeared to have no significant effect. The largest effect QTL, m2b-1, was detected only in the LCa background; it had an estimated LOD value of 8.34 (permutation test: $P < 0.0001$), based on a genome-wide 5% LOD threshold of 2.25. In the Lld background, LG 2 had no detectable effect. Allelic dominance was thus confirmed for major QTL m2b-1 (cf. Fig. 4b, e).

Table 2. Effects of linkage groups harbouring major QTL in the 'clonal' sibship analysis

LCa background					Lld background				
		LG 1		Difference†		LG 1		Difference†	
		Ca	Id			Ca	Id		
LG 2b	Ca	22.6 (28)	42.8 (18)	+18.2*	LG 2b	Ca	61.7 (16)	67.1 (13)	+2.0
	Id	53.6 (26)	66.1 (29)	+13.7**		Id	70.3 (9)	67.2 (9)	+4.2
Difference		+26.1***	+22.3***		Difference	+5.5	+3.6		
		LG 5		Difference		LG 5		Difference	
LG 2b	Ca	44.4 (16)	24.1 (24)	-18.5*	LG 2b	Ca	74.9 (13)	55.9 (19)	-14.4**
	Id	65.1 (24)	52.6 (14)	-9.2		Id	69.0 (22)	55.4 (14)	-16.0**
Difference		+19.4**	+31.4***		Difference	+4.7	+3.5		
		LG 1		Difference		LG 1		Difference	
LG 5	Ca	45.0 (21)	62.6 (21)	+17.6*	LG 5	Ca	67.2 (14)	59.0 (16)	+4.0
	Id	31.9 (23)	51.5 (19)	+18.1*		Id	64.0 (17)	51.4 (16)	-13.3**
Difference		-9.8	-9.6		Difference	-2.0	-18.7***		

For each of the two maternal genetic backgrounds the sibship data set is partitioned by the genotype of nonrecombined linkage groups (LG). Effects of and interactions between LG 1, 2b and 5, harbouring major QTL, are shown. Female acceptances of a *N. vitripennis* male were arcsine square-root transformed and class medians for the sibships (number in parentheses) in each genotype class are tabulated.

†Point estimates for the difference in mate acceptance between genotype classes, together with the direction of the QTL effect (+ = increased, - = decreased acceptance of a *N. vitripennis* male), and significance after Bonferroni correction (Wilcoxon–Mann–Whitney test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

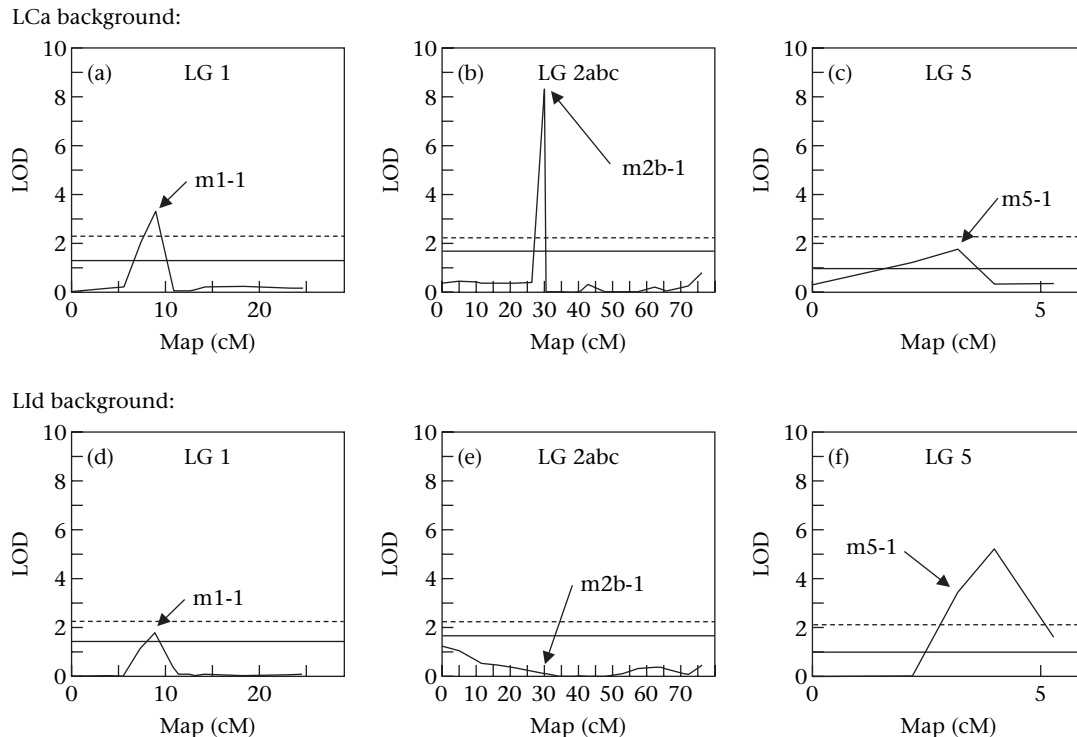


Figure 4. MQM mapping of major QTL in the LCa maternal genetic background (a, b, c) and the LId genetic background (d, e, f) of the 'clonal' sibship analysis in the 2-h mating assay. Only linkage groups with major QTL linked to markers m1-1 (a, d), m2b-1 (b, e) and m5-1 (c, f) are shown. Map position is indicated on the X axis. Likelihood odds (LOD) scores for presence of a QTL are shown on the Y axis. A permutation test implemented in MapQTL gave 0.05 significance thresholds for each linkage group (solid line) and for genome-wide (dashed line) LOD scores of QTL.

The second largest effect QTL, m5-1, was highly significant only in the LId genetic background (LOD = 5.22, genome-wide threshold = 2.15, $P < 0.0001$). A marginally significant effect of LG 5 was detected in the LCa background once the genotype of m2b-1 was controlled for (LOD = 1.79, linkage group LOD threshold = 1.05, $P = 0.021$). We were not able to determine whether, for example, LG 5 harbours a single major QTL with co-dominant gene expression, or whether, perhaps, multiple QTL exist on this linkage group. Our results did indicate, however, that it was the LCa alleles on this linkage group that reduced the level of sexual isolation between *N. longicornis* and *N. vitripennis*, and not LId alleles (as was the case for major QTL m1-1 and m2b-1).

Finally, for LG 1, only a single major QTL, m1-1, could be detected; existence of a second QTL (m1-2) nearby could not be confirmed (nor ruled out). The effect of m1-1 was highly significant in the LCa background (LOD value = 3.31, genome-wide threshold = 2.35, $P < 0.0001$), with the LId allele increasing mate acceptance. The same genetic region on LG 1 may have an effect in the other (LId) background (LOD = 1.77, linkage group LOD threshold = 1.45, $P = 0.029$), with the LCa/LId heterozygotes having slightly increased acceptance relative to LId/LId homozygotes in this region. Multiple QTL could exist in this region and may explain the previously mentioned change in the direction of effects of LG 1 between the two maternal genetic backgrounds (cf. Table 2).

Follow-up Behavioural and Genetic Studies

To test specific hypotheses resulting from our QTL analysis, we constructed a set of recombinant inbred lines (RILs) as described in methods. Thirty-six RILs were tested for their female mate acceptance of *N. vitripennis* males using the 2-h assay; females from particular RILs were either LCa-like, intermediate, LId-like, or transgressive in their mate acceptance (data not shown).

Our analysis of RILs supports the finding that the major QTL m2b-1 significantly increased acceptance of *N. vitripennis* males in the 2-h mating assay (Wilcoxon–Mann–Whitney test: $W = 15.0$, $P < 0.0007$; Table 3). The

Table 3. Effect of major QTL m2b-1 in recombinant inbred lines

Time of assay	Ca/Ca	N	Ld/Ld	N	Difference†	P
First courtship	9.1	5	12.9	14	+9.1	0.1144
2 h	9.1	5	34.8	14	+26.4	<0.0007

Female acceptance of a *N. vitripennis* male in the first courtship, and during 2 h, in LCa/LId recombinant inbred lines (RILs).

Class medians of transformed proportions mate acceptance for RILs with a Ca/Ca, or a Ld/Ld genotype at marker m2b-1, and the number of RILs per genotype class (N) are shown. Numbers of females assayed for each recombinant inbred line were 40–60 for the first courtship, and 50–80 for the 2-h assay.

†Point estimates for the difference between classes, and P-value (Wilcoxon–Mann–Whitney test) are indicated.

available data for major QTL m5-1 were still very limited, but seem to suggest that it may have no independent effect in an otherwise homozygous LCa/LCa background. Furthermore, when major QTL m2b-1 (with the largest effect in the LCa background) was homozygous Lld/Lld, a significant effect of major QTL m5-1 was detected (Wilcoxon–Mann–Whitney test: $W = 38.0$, $P = 0.0184$), but in the opposite direction than expected from the sibship analysis. Lld/Lld lines for m5-1 had higher acceptance than LCa/LCa lines. Three possible explanations are: (1) recombination between the actual acceptance QTL and marker m5-1 occurred during construction of the RILs; (2) differential effects of the LG 5 major QTL occur when heterozygous versus when homozygous; or (3) there exists complex epistasis involving this QTL that was not revealed in the earlier analysis. We have not yet resolved these alternatives.

We also conducted a 15-min observation assay on 19 recombinant inbred lines to investigate the behavioural basis of our largest effect major QTL m2b-1. Our results suggest that this QTL had no effect on female acceptance during the first courtship (Table 3). However, its large effect on cumulative acceptance across multiple courtships in the 2-h mating assay was confirmed (Table 3). We therefore conclude that this QTL may be involved in a behavioural mechanism for continued resistance of LCa to *N. vitripennis* males during repeated courting.

Finally, we examined this subset of recombinant inbred lines for female mate acceptance in the first and second courtship, and over a 15-min time interval (Fig. 5). Many lines showed a low acceptance of a *N. vitripennis* male in the first courtship, similar to that found in both parental lines. However, several lines showed an increased acceptance at the first courtship, similar to or even significantly higher than that observed in F1 females. These results indicate that the RILs had segregated for major effect QTL for mate acceptance in the first courtship. This was confirmed by the observation that some of these lines had relatively small chromosomal regions from the Lld strain in an otherwise LCa background.

Certain RILs showed increased cumulative acceptance by the second courtship and after 15 min (see Fig. 5), similar to the Lld strain. It is likely that some of the QTL identified in the 2-h assay were also involved in mate discrimination in the first and second courtships, although this remains to be identified. However, we have shown that major QTL m2-b1 does not affect acceptance in the first courtship, but does appear to be responsible for the increased acceptance across multiple courtships (Table 3, Fig. 5).

DISCUSSION

Key findings of this study were: (1) the two *N. longicornis* strains differed in female acceptance of heterospecific males, with one strain initially showing mate discrimination but then significantly increasing its mate acceptance in subsequent courtships, and the other strain consistently maintaining resistance to heterospecific matings; (2) the mate acceptance differences had a relatively simple

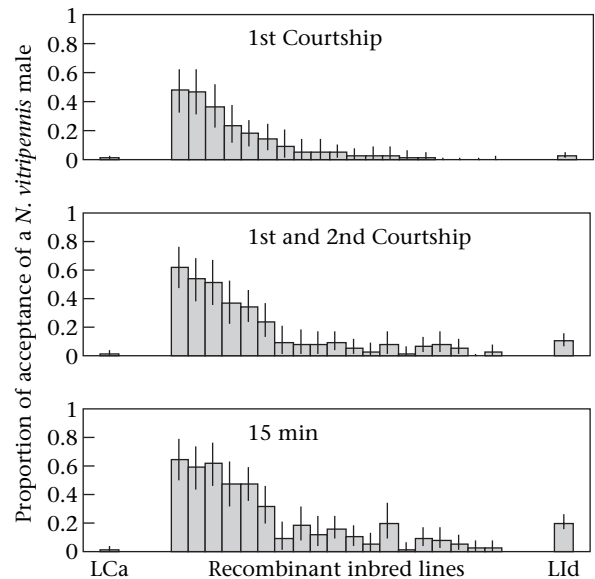


Figure 5. Proportions of female mate acceptance of a heterospecific *N. vitripennis* male in the 15-min behavioural observation assay of hybrid LCa/Lld recombinant inbred lines. Nineteen lines are shown (with 95% confidence limits) in order of their female mate acceptance in the first courtship. Several of these lines showed a dramatically increased acceptance at the first courtship, more so than did F1 females, whereas other lines resembled either LCa females (on the left) or Lld females (on the right), or were intermediate in their mate acceptance behaviour(s) across multiple courtships. Sample sizes ranged from 33 to 82 (mean = 51) females per inbred line tested.

genetic basis, consisting of but a few major QTL and their interactions; (3) major QTL alleles that increased mate acceptance appeared to be dominant relative to discrimination alleles; (4) our largest effect major QTL appeared to be involved in the differential increase in acceptance of heterospecific males during multiple courtships; (5) F1 interstrain females and several recombinant inbred lines showed dramatically elevated acceptance of *N. vitripennis* males in the first courtship, even though both parental strains showed low acceptance in the first courtship. These results provide some insights in the genetic and phenotypic architecture of female mate discrimination in *Nasonia*, although much remains to be revealed.

The finding of relatively few genomic regions of large effect on female mate acceptance is in contrast to a more polygenic genetic architecture that has been observed in some other cases, such as in the Hawaiian cricket genus *Laupala* (e.g. Shaw & Parsons 2002) or in *Drosophila* (see Hollocher 1998). It is likely that, with increased size of the population used for mapping and QTL analysis, additional minor QTL may be found. The sizes of the effects of major QTL, as found in our sibship analysis, were overestimates (Tanksley 1993; Beavis 1998), and can be estimated more accurately from introgressions of individual QTL. By this method we were able to confirm a major QTL on linkage group 2 (m2b-1) that increased acceptance of *N. vitripennis* males by around 26%. Minor QTL that are suggested in our data set, but that could not be confirmed, may be

found to exert significant effects, possibly through epistatic interactions.

Male haploidy was used to generate large numbers of genetically identical females, which reduces stochastic noise associated with measuring mate preference. Two populations were constructed for QTL analysis, which shared unique paternal sets of chromosomes and differed with respect to the maternal set (LCa or LId). Thus, we were able to directly apply information about QTL found in one genetic background in the QTL analysis of the other genetic background. This not only increased our resolution to detect QTL, but also enabled us to better distinguish putative QTL from false positives that arise from cosegregation of QTL on other linkage groups. In addition, significant epistatic interactions between major QTL were uncovered. Subsequent introgression of major QTL into the LCa background will facilitate the search for additional QTL. More importantly, it enables us to assess the effects of individual QTL more accurately, as well as to study the genetic interactions within and between loci of interest, within a controlled genetic background.

An important observation is that the 'discrimination alleles' at all three major QTL are recessive, and the same has been found for female mate preference QTL involved in acceptance between *N. vitripennis* and *N. giraulti* (B.-J. Velthuis & J. H. Werren, unpublished data). One interpretation of our results is that the recessive discrimination alleles are 'hypomorphs', showing a reduction or loss of function at the locus, and reduced function in a particular pathway. If correct, this would imply that the products of the major QTL in our study function to increase acceptance and hypomorphs therefore decrease acceptance.

Behavioural characterizations of female mate discrimination in the two *N. longicornis* strains and cross lines indicate that both strains are initially reluctant to mate with *N. vitripennis* males, but that LCa females remain resistant whereas LId females become receptive with subsequent courtships. The major QTL on LG 2 appears to be involved in this difference in 'choosiness' (see Jennions & Petrie 1997) between LCa and LId, since it affects mate acceptance after 2 h but not in the initial courtship. It remains to be determined whether LId females habituate to aversive stimuli emanating from this heterospecific male, or whether, for example, in the absence of a relevant conspecific signal of mate quality, other signals of mate quality, for which the two species have not diverged, come to play a more prominent role (for a discussion of this point see Boake 2000; Ptacek 2000). Alternatively, the LId threshold for mate acceptance may simply decline with time, independently of prior experience. Finally, it may be that excitatory stimuli from *N. vitripennis* males (e.g. pheromones, see Barrass 1960; van den Assem 1976; van den Assem et al. 1980), which are initially insufficient to induce receptivity, accumulate over time and result in acceptance by LId females in later courtships. These alternatives have not yet been resolved.

Female mate preference genes are probably involved in sensory and neurological mechanisms that process excitatory or inhibitory courtship signals emanating from the male. The preference genes may modulate the behavioural response by the female through signal-level thresholds in

these mechanisms. Males tend to differ within as well as between species, for example, in age, nutritional state, recent courtship and mating experiences, and so forth; in short, in various aspects of their courtship performance. Therefore, the behavioural plasticity of the female mate discrimination mechanism may actually function by 'appropriately' adjusting its response thresholds during courtship to ensure mating eventually does take place (and with the better of available mates). Alternatively, some form of stimulus filtering may be taking place, in which the effects of 'secondary' stimuli (e.g. body size, thoracic muscle vibrations, or simply a male's persistence; van den Assem 1976; van den Assem & Putters 1980; van den Assem & Jachmann 1999) depend on the presence or absence of the proper sign stimulus (i.e. male mandibular gland pheromones; van den Assem et al. 1980). We postulate the major QTL on LG 2 to be a locus involved in modulation, that is, the changing of some response threshold, because it specifically affects the level of resistance to *N. vitripennis* males over repeated courtships.

The observation that F1 females showed dramatically higher acceptance of *N. vitripennis* males in the first courtship than either parental strain is consistent with there being a threshold for acceptance and dominance of acceptance alleles. A threshold is implied by this finding because a more graded response would not result in the dramatic increase of acceptance from around 2% in both parental strains to 15–29% in F1 females. The result can be explained if both strains contain acceptance alleles but with an expression at an insufficient level to stimulate acceptance in the first courtship; F1 females carry dominant acceptance alleles from both strains, resulting in a surpassing of the threshold for acceptance in the first courtship. Consistent with this view, we detected acceptance alleles in both LId and LCa.

Clearly, more behavioural studies are needed to map the genetic architecture onto the behavioural architecture of female preference in *Nasonia*. We currently do not know the species specific male courtship signals that are involved in mate acceptance, although previous studies suggest that male mandibulatory gland pheromones are important in intraspecific mate acceptance (van den Assem et al. 1980). In addition, the males of all three species differ in aspects of their courtship behaviour (van den Assem & Werren 1994) and morphology (Darling & Werren 1990), factors that could be important in heterospecific mate discrimination. One nice feature of *Nasonia* is that we can use the variation in female mate acceptance to introgress male traits from *N. vitripennis* that LId females find acceptable but that LCa females discriminate against. Thus, we have the potential to allow the wasps to 'tell us' by these genetic experiments which cues are important. Such introgressions are now underway.

N. longicornis and *N. vitripennis* cooccur in bird nests at appreciable frequencies (Darling & Werren 1990). Therefore, the opportunities for interspecies matings may be significant in nature. Hybrid matings suffer reduced fitness both by endosymbiotic *Wolbachia*, which cause a postmating incompatibility (Breeuwer & Werren 1990; Bordenstein et al. 2001), and by reduced fitness of hybrids surviving the incompatibility (Breeuwer & Werren 1995; Gadau et al.

1999). This would impose a significant cost to females who mate heterospecifically and therefore select for discriminating alleles at mate preference loci. It remains to be determined whether the exposure of *N. longicornis* females to *N. vitripennis* males differs geographically, possibly explaining variation in mate discrimination. If interspecies copulations occur in nature, they are most likely to involve the first or second courtships only. Most *Nasonia* females are rapidly mated by conspecific males if such are available, and will leave the mating arena shortly after. Therefore, acceptance in the first and second courtship is biologically most relevant to interspecies mating in nature. Although our two analysed strains showed extremely low acceptance of *N. vitripennis* males in the first courtship, the F1 interstrain females showed significant levels of mating with heterospecific males in the first courtship. This indicates that natural populations are segregating for genetic variation in this trait. Due to its biological relevance to premating isolation, we are now conducting more detailed studies of the genetic and behavioural basis of mate discrimination in the first courtship.

Our results also have general implications for evolutionary processes involving mate choice, including models of runaway sexual selection, reinforcement and sympatric speciation. Theoretical models indicate that female mate discrimination may lead to rapid evolution of sexual isolation. It does so by increasing the magnitude of the indirect association between mate preference and fitness, initially established by genetic drift or selection (Lande 1981; Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999; Geritz & Kisdi 2000). Comparison of simulation results from these polygenic models suggests that a relatively low number of mate discrimination loci facilitate evolution of sexual isolation. Other simulation studies show that sympatric speciation can result from sexual selection acting via only a few, major effect, genes (Turner & Burrows 1995; Payne & Krakauer 1997, 2000; Higashi et al. 1999; Takimoto et al. 2000; Takimoto 2002). A common thread in these models may be that the initial rate at which the association between mate preference and fitness evolves is important for the likelihood of speciation, and this, in turn, is enhanced when relatively few genes of large effect are involved as opposed to many genes of small effect. In conclusion then, the underlying genetic architecture strongly influences how easily the association between female mate discrimination and fitness may initially arise, and subsequently increase in magnitude. A fairly simple genetic basis of female mate discrimination, such as found in our study, may be particularly well suited towards the evolution of premating isolation or for the rapid evolution of mate recognition signals by sexual selection.

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References

- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Applied Biosystems 1998. *Genescan Analysis Software. Version 3.1. User's Manual*. Boston, Massachusetts: Perkin-Elmer.
- van den Assem, J. 1976. Male courtship behaviour, female receptivity signal, and size differences between sexes in Pteromalinae (Hym. Chalcidoidea Pteromalidae), and comparative notes on other chalcidoids. *Netherlands Journal of Zoology*, **26**, 535–548.
- van den Assem, J. & Jachmann, F. 1999. Changes in male perseverance in courtship and female readiness to mate in a strain of the parasitic wasp *Nasonia vitripennis* over a period of 20+ years. *Netherlands Journal of Zoology*, **49**, 125–137.
- van den Assem, J. & Putters, F. A. 1980. Patterns of sound produced by courting chalcidoid males and its biological significance. *Entomologica Experientia et Applicata*, **27**, 293–302.
- van den Assem, J. & Werren, J. H. 1994. A comparison of the courtship and mating behaviour of 3 species of *Nasonia*. *Journal of Insect Behavior*, **7**, 53–66.
- van den Assem, J., Jachmann, F. & Simbolotti, P. 1980. Courtship behaviour of *Nasonia vitripennis*: some qualitative, experimental evidence for the role of pheromones. *Behaviour*, **75**, 301–307.
- Bakker, T. C. M. & Pomiankowski, A. 1995. The genetic basis of female mate preferences. *Journal of Evolutionary Biology*, **8**, 129–171.
- Barrass, R. 1960. The courtship behavior of *Mormoniella vitripennis* (Hym. Pteromalidae). *Behaviour*, **15**, 185–209.
- Beavis, W. D. 1998. QTL analyses: power, precision and accuracy. In: *Molecular Dissection of Complex Traits* (Ed. by A. H. Paterson), pp. 145–162. Boca Raton, Florida: CRC Press.
- Bender, R. & Lange, S. 2001. Adjusting for multiple testing – when and how? *Journal of Clinical Epidemiology*, **54**, 343–349.
- Benham, J. 2001. *Genographer*. Version 1.6.0. <http://hordeum.oscs.montana.edu/genographer>.
- Beukeboom, L. W. & van den Assem, J. 2001. Courtship and mating behaviour of interspecific *Nasonia* hybrids (Hymenoptera, Pteromalidae): a grandfather effect. *Behavior Genetics*, **31**, 167–177.
- Bland, J. M. & Altman, D. G. 1995. Multiple significance tests: the Bonferroni method. *British Medical Journal*, **310**, 170.
- Blum, M. S., Blum, N. A. (Eds) 1979. *Sexual Selection and Reproductive Competition in Insects*. New York: Academic Press.
- Boake, C. R. B. (Ed) 1994. *Quantitative Genetic Studies of Behavioral Evolution*. Chicago: University of Chicago Press.
- Boake, C. R. B. 2000. Flying apart: mating behaviour and speciation. *BioScience*, **50**, 501–508.
- Boake, C. R. B., DeAngelis, M. P. & Andreadis, D. K. 1997. Is sexual selection and species recognition a continuum? Mating behaviour of the stalk-eyed fly *Drosophila heteroneura*. *Proceedings of the National Academy of Sciences, U.S.A.*, **94**, 12442–12445.
- Bordenstein, S. R., Drapeau, M. D. & Werren, J. H. 2000. Intraspecific variation in sexual isolation in the jewel wasp *Nasonia*. *Evolution*, **54**, 567–573.

- Bordenstein, S. R., O'Hara, F. P. & Werren, J. H. 2001. *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature*, **409**, 707–710.
- Bradbury, J. W., Andersson, M. B. (Eds) 1987. *Sexual Selection: Testing the Alternatives*. New York: J. Wiley.
- Breeuwer, J. A. J. & Werren, J. H. 1990. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature*, **346**, 558–560.
- Breeuwer, J. A. J. & Werren, J. H. 1995. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. *Evolution*, **49**, 705–717.
- Carracedo, M. C., Suarez, B., Asenjo, A. & Casares, P. 1998. Genetics of hybridization between *Drosophila simulans* females and *D. melanogaster* males. *Heredity*, **80**, 17–24.
- Chase, K., Adler, F. R. & Lark, K. G. 1997. Epistat: a computer program for identifying and testing interactions between pairs of quantitative trait loci. *Theoretical and Applied Genetics*, **94**, 724–730.
- Choe, J. C., Crespi, B. J. (Eds) 1997. *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge: Cambridge University Press.
- Churchill, G. A. & Doerge, R. W. 1994. Empirical threshold values for quantitative trait mapping. *Genetics*, **138**, 963–971.
- Cosse, A. A., Campbell, M. G., Glover, T. J., Linn, C. E., Todd, J. L., Baker, T. C. & Roelofs, W. L. 1995. Pheromone behavioural responses in unusual male European corn borer hybrid progeny not correlated to electrophysiological phenotypes of their pheromone-specific antennal neurons. *Experientia*, **51**, 809–816.
- Coyne, J. A. 1989. Genetics of sexual isolation between two sibling species, *Drosophila simulans* and *Drosophila mauritiana*. *Proceedings of the National Academy of Sciences, U.S.A.*, **86**, 5464–5468.
- Daniel, W. W. 1999. *Biostatistics: a foundation for Analysis in the Health Sciences*. 7th edn. New York: J. Wiley.
- Darling, D. Ch. & Werren, J. H. 1990. Biosystematics of *Nasonia* (Hymenoptera: Pteromalidae): two new species reared from birds' nests in North America. *Annals of the Entomological Society of America*, **83**, 352–370.
- Dieckmann, U. & Doebeli, M. 1999. On the origin of species by sympatric speciation. *Nature*, **400**, 354–357.
- Doi, M., Matsuda, M., Tomaru, M., Matsubayashi, H. & Oguma, Y. 2001. A locus for female discrimination behavior causing sexual isolation in *Drosophila*. *Proceedings of the National Academy of Sciences, U.S.A.*, **98**, 6714–6719.
- Foster, S. A. & Endler, J. A. 1999. *Geographic Variation in Behavior. Perspectives on Evolutionary Mechanisms*. New York: Oxford University Press.
- Gadau, J., Page, R. E. & Werren, J. H. 1999. Mapping of hybrid incompatibility loci in *Nasonia*. *Genetics*, **153**, 1731–1741.
- Gadau, J., Page, R. E. & Werren, J. H. 2002. The genetic basis of the interspecific differences in wing size in *Nasonia* (Hymenoptera; Pteromalidae): major quantitative trait loci and epistasis. *Genetics*, **161**, 673–684.
- Geritz, S. A. H. & Kisdi, E. 2000. Adaptive dynamics in diploid, sexual populations and the evolution of reproductive isolation. *Proceedings of the Royal Society of London, Series B*, **267**, 1671–1678.
- Gokhman, V. E. & Westendorf, M. 2000. The chromosomes of three species of the *Nasonia* complex (Hymenoptera, Pteromalidae). *Beitrage Entomologie, Supplement*, **50**, 193–198.
- Hall, J. C. 1994. The mating of a fly. *Science*, **264**, 1702–1714.
- Heisler, I. L. 1984. Inheritance of female mating propensities for yellow locus genotypes in *Drosophila melanogaster*. *Genetical Research*, **44**, 133–149.
- Higashi, M., Takimoto, G. & Yamamura, N. 1999. Sympatric speciation by sexual selection. *Nature*, **402**, 523–526.
- Hollocher, H. 1998. Reproductive isolation in *Drosophila*: how close are we to untangling the genetics of speciation? *Current Opinion in Genetics & Development*, **8**, 709–714.
- Hollocher, H., Ting, C.-T., Wu, M.-L. & Wu, C.-I. 1997. Incipient speciation by sexual isolation in *Drosophila melanogaster*: extensive genetic divergence without reinforcement. *Genetics*, **147**, 1191–1201.
- Iwasa, Y., Pomiankowski, A. & Nee, S. 1991. The evolution of costly mate preferences II. The "handicap" principle. *Evolution*, **45**, 1431–1442.
- Jansen, R. C. 1993. Interval mapping of multiple quantitative trait loci. *Genetics*, **135**, 205–211.
- Jansen, R. C. & Stam, P. 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, **136**, 1447–1455.
- Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews of the Cambridge Philosophical Society*, **72**, 283–327.
- Kelly, J. K. & Noor, M. A. F. 1996. Speciation by reinforcement: a model derived from studies of *Drosophila*. *Genetics*, **143**, 1485–1497.
- Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. *Evolution*, **36**, 1–12.
- Kirkpatrick, M. & Ravné, V. 2002. Speciation by natural and sexual selection: models and experiments. *American Naturalist, Supplement*, **159**, 22–35.
- Kirkpatrick, M. & Servedio, M. R. 1999. The reinforcement of mating preferences on an island. *Genetics*, **151**, 865–884.
- Klun, J. A., Chapman, O. L., Mattes, K. C., Wojtkowski, P. W., Beroza, M. & Sonnet, P. E. 1973. Insect sex pheromones: minor amount of opposite geometrical isomer critical to attraction. *Science*, **181**, 661–663.
- Kondrashov, A. S. & Kondrashov, F. A. 1999. Interactions among quantitative traits in the course of sympatric speciation. *Nature*, **400**, 351–354.
- Landé, R. 1981. Models of speciation by sexual selection and adaptation on polygenic traits. *Proceedings of the National Academy of Sciences, U.S.A.*, **78**, 3721–3725.
- Lander, E. S. & Botstein, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, **121**, 185–199.
- Lander, E. S. & Green, P. 1987. Construction of multilocus genetic linkage maps in humans. *Proceedings of the National Academy of Sciences, U.S.A.*, **84**, 2363–2367.
- Lander, E., Green, P., Abrahamson, J., Barlow, A., Daly, M., Lincoln, S. & Newburg, L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, **1**, 174–181.
- Lewontin, R. C. 1974. *The Genetic Basis of Evolutionary Change*. New York: Columbia University Press.
- Liou, L. W. & Price, T. D. 1994. Speciation by reinforcement of premating isolation. *Evolution*, **48**, 1451–1459.
- Liu, B. H. 1998. *Statistical Genomics: Linkage, Mapping and QTL Analysis*. Boca Raton, Florida: CRC Press.
- Löfstedt, C., Hansson, B. S., Roelofs, W. & Bengtsson, B. O. 1989. No linkage between genes controlling female pheromone production and male pheromone response in the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera, Pyralidae). *Genetics*, **123**, 553–556.
- Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sunderland, Massachusetts: Sinauer.
- Mueller, U. G. & Wolfenbarger, L. L. 1999. AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution*, **14**, 389–394.

- Noor, M. A. F., Grams, K. L., Bertucci, L. A. & Reiland, J. 2001. Chromosomal inversions and the reproductive isolation of species. *Proceedings of the National Academy of Sciences, U.S.A.*, **98**, 12084–12088.
- O'Donald, P. 1979. *Genetic Models of Sexual Selection*. Cambridge: Cambridge University Press.
- Olsen, J. M. & Boehnke, M. 1990. Monte Carlo comparison of preliminary methods for ordering multiple genetic loci. *American Journal of Human Genetics*, **47**, 470–482.
- van Ooijen, J. W. & Voorrips, R. E. 2001. *JoinMap 3.0. Software for the Calculation of Genetic Linkage Maps*. Wageningen: Plant Research International.
- van Ooijen, J. W., Boer, M. P., Jansen, R. C. & Maliepaard, C. 2002. *MapQTL 4.0. Software for the Calculation of QTL Positions on Genetic Maps*. Wageningen: Plant Research International.
- Payne, R. J. H. & Krakauer, D. C. 1997. Sexual selection, space and speciation. *Evolution*, **51**, 1–9.
- Payne, R. J. H. & Krakauer, D. C. 2000. Disruptive sexual selection. *Trends in Ecology and Evolution*, **15**, 419–420.
- Pomiankowski, A., Iwasa, Y. & Nee, S. 1991. The evolution of costly mate preferences I. Fisher and biased mutation. *Evolution*, **45**, 1422–1430.
- Ptacek, M. B. 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behavioural Processes*, **51**, 111–134.
- Ritchie, M. G. 1992a. Setbacks in the search for mate-preference genes. *Trends in Ecology and Evolution*, **7**, 328–329.
- Ritchie, M. G. 1992b. Behavioral coupling in tettigoniid hybrids (Orthoptera). *Behavior Genetics*, **22**, 369–379.
- Roupe van der Voort, J. N. A. M., van Zandvoort, P., van Eck, H. J., Folkertsma, R. T., Hutten, R. C. B., Draaistra, J., Gommers, F. J., Jacobsen, E., Helder, J. & Bakker, J. 1997. Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. *Molecular and General Genetics*, **255**, 438–447.
- Ryan, M. J. 1985. *The Tungara Frog: a Study in Sexual Selection and Communication*. Chicago: University of Chicago Press.
- Scott, D. 1994. Genetic variation for female mate discrimination in *Drosophila melanogaster*. *Evolution*, **48**, 112–121.
- Shaw, K. L. 2000. Interspecific genetics of mate recognition: inheritance of female acoustic preference in Hawaiian crickets. *Evolution*, **54**, 1303–1312.
- Shaw, K. L. & Parsons, Y. M. 2002. Divergence of mate recognition behavior and its consequences for genetic architectures of speciation. *American Naturalist, Supplement*, **159**, 61–75.
- Siegel, S. & Castellan, N. J., Jr. 1989. *Nonparametric Statistics for the Behavioural Sciences*. 2nd edn. New York: McGraw-Hill.
- Sokal, R. R. & Rohlf, F. J. 1998. *Biometry: the Principles and Practice of Statistics in Biological Research*. 3rd edn. New York: W.H. Freeman.
- Stringham, H. M. & Boehnke, M. 2001. LOD scores for gene mapping in the presence of marker map uncertainty. *Genetic Epidemiology*, **21**, 31–39.
- Takimoto, G. 2002. Polygenic inheritance is not necessary for sympatric speciation by sexual selection. *Population Ecology*, **44**, 87–91.
- Takimoto, G., Higashi, M. & Yamamura, N. 2000. A deterministic model for sympatric speciation by sexual selection. *Evolution*, **54**, 1870–1881.
- Tanksley, S. D. 1993. Mapping polygenes. *Annual Review of Genetics*, **27**, 205–233.
- Thornhill, R. & Alcock, J. 1983. *The Evolution of Insect Mating Systems*. Cambridge, Massachusetts: Harvard University Press.
- Tregenza, T. & Butlin, R. K. 1999. Speciation without isolation. *Nature*, **400**, 311–312.
- Turner, G. F. & Burrows, M. T. 1995. A model of sympatric speciation by sexual selection. *Proceedings of the Royal Society of London, Series B*, **260**, 287–292.
- Voorrips, R. E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. *Heredity*, **93**, 77–78.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuijper, M. & Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.
- Weston, R. F., Qureshi, I. & Werren, J. H. 1999. Genetics of wing size differences between two *Nasonia* species. *Journal of Evolutionary Biology*, **12**, 586–595.
- West-Eberhard, M. J. 1984. Sexual selection, competitive communication and species specific signals in insects. In: *Insect Communication* (Ed. by T. Lewis), pp. 283–324. New York: Academic Press.
- Wilkinson, G. S. & Reillo, P. R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proceedings of the Royal Society of London, Series B*, **255**, 1–6.
- Yamamoto, D., Jallon, J.-M. & Komatsu, A. 1997. Genetic dissection of sexual behaviour in *Drosophila melanogaster*. *Annual Review of Entomology*, **42**, 551–585.
- Zahavi, A. 1975. Mate selection – a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205–214.
- Zhu, J. W., Löfstedt, C. & Bengtsson, B. O. 1996. Genetic variation in the strongly canalized pheromone communication system of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Genetics*, **144**, 757–766.