The genetics of species differences within the genus *Nasonia* ASHMEAD 1904 (Hymenoptera: Pteromalidae)

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Christof Pietsch

aus Laubach

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Eingereicht am:

1. Gutachter: Privatdozent Dr. J. Gadau

2. Gutachter: Prof. Dr. E. Buchner

1. Prüfer: Privatdozent Dr. J. Gadau

2. Prüfer: Prof. Dr. E. Buchner

Tag der mündlichen Prüfung:

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Introduction

Investigating the genetics of species differences assumes that species are real entities and are not simply delineated by subjective classification.

Dobzhansky assertained that "Discrete groups are encountered among animals as well as plants, in those that are structurally simple as well as in those thar are very complex. Formation of discrete groups is so nearly universal that it must be regarded as a fundamental characteristic of organic diversity" (Dobzhansky 1937). Indeed, often a one-to-one correspondence exists between the scientific delineation of species and popular denomination of species (Mayr 1963, Diamond 1966, Bulmer and Tyler 1968, Berlin 1973, Bulmer et al. 1975). However, the strongest evidence for the existence of species comes from the existence of sexually reproducing groups living in sympatry separated by phenotypic and genetic gaps (Coyne and Orr 1998).

Dobzhansky (1937) and Mayr (1942) introduced the biological species concept (BSC) defining species as "[...] groups of actually or potentially interbreeding natural populations, which are reproductively, isolated from other such groups" (Mayr 1942, p. 120). Thus, the integrity of a species is maintained by isolation factors leading to reproductive isolation (RI). The mechanisms of RI can be divided into those that act before (prezygotic) and that after fertilisation (postzygotic) (see Dobzhansky 1970, Coyne and Orr 2004). Prezygotic isolating factors comprise e.g. behavioural, ecological, mechanical isolation factors that prevent hybridisation, whereas postzygotic isolating factors refer to hybrid sterility or inviability.

Species differences between closely related taxa often involve sexual dimorphic traits (e.g. in *Drosophila* (Ringo 1977) or in birds (Barraclough et al. 1995), that often coincide with preproductive isolation (Lande 1982, Gavrilets 2000, Via 2001, references in Panhuis et al. 2001). Coyne and Orr (1989a, 1997) found in comparative studies that sexual isolation in *Drosophila* species-pairs were consistently far stronger in sympatry than allopatry suggesting

that current sympatric *Drosohila* species may not coexist without mate-discrimination. Formal models have demonstrated that sexual selection can lead to reproductive isolation in sympatry by generating strong assortative mating e.g. through female choice (Van Doorn et al. 1998, Higashi et al. 1999, Takimoto et al. 2000, Takimoto 2002), mutual mate choice (Almeida and Vistulo de Abreu 2003), sexual conflict (Gavrilets and Waxman 2002), sex-ratio selection (Lande et al. 2001) or intra-sexual selection (Wiernasz and Kingsolver 1992).

It is often assumed that females prefer traits of conspecific males and this in turn cause ethological isolation. However, divergent sexual selection need not inevitably cause substantial reproductive isolation. For instance, the sexual selected shape of tailfins in *Xiphophorus helleri* and *X. macualtus* (Basolo 1990, 1995), or the forehead crests in species of the auklet genus *Aethia* (Jones and Hunter 1998) do not cause ethological isolation (see also Ryan 1998). Thus, secondary sexual traits may be involved in reproductive isolation or may resemble species differences that evolved as a by-product of reproductive isolation (see Orr 2001).

The genetics of species differences

One of the most fundamental questions in evolutionary biology is about the genetic architecture of phenotypic divergence between species. This global issue is concerned about the number of genes, the distribution of their phenotypic effects, their location in the genome and the role of epistasis in phenotypic evolution. On of the most contentious debates was concerned about the distribution of effects. Darwin was convinced that selection act upon many genes with small effects leading to divergence, resulting in a gradual evolutionary process. The first mathematical treatise of this idea was done by Fisher (1930), who introduced the infinitesimal model, which assumes a completely fluid and coherent variation produced by mutations. It has been argued that major mutations likely exert deleterious pleiotropic side effects in an n-dimensional phenotype and thus may disrupt the integrity of a

complex organism. But Maynard Smith et al. (1985) asserted that deleterious side effects of major mutations may be attenuated by modifier genes. Orr (1998, 1999) inferred from simulations that the phenotypic effects of alleles that become fixed during adaptation follow an exponential distribution.

But caution is necessary in the interpretation of the magnitude of effects. It is worth noting that the phenotypic effect of a locus depends crucial on the referred measure. A gene effect may be either estimated in reference to the phenotypic species gap, or to the standing phenotypic variation within species. For instance, a single gene may explain 100 % of the species gap, but may has a small contribution to the standing phenotypic variation (for details see Orr 2001, True et al. 1997). Additionally, the magnitude in terms of the species gap (equivalent to the explained variance in QTL analysis) is a time variant measure. The first mutation that gets fixed explains 100 % of the phenotypic species gap, but explains increasingly less with every new mutation that contributes to trait divergence (Orr 2001). These considerations were often left disregarded and may at least partially explain the inconsistency of empirical data upon the genetics of species differences. However, Orr (2001) stated, "that there is, of course, no fundamental reason why the number of factors should show any consistency; every case of divergence might differ from every other, reflecting differences in the strength of selection, the nature of the standing genetic variation, and so on".

With the advent of molecular markers and the development of statistical machinery in QTL (quantitative trait locus) mapping, there is growing evidence, that in many cases the genetic architecture of phenotypic evolution can often be accounted for by few loci with large effects (e.g. divergence of teosinte and maize (Edwards et al. 1987, Doebley (1992), blossom characters in *Mimulus* (Lin and Ritland 1997), fruit characters in tomato (Grandillo and Tanksley 1996), wing size in *Nasonia* (Gadau et al. 2002), courthisp song in *Drosophila* (Huttunen et al. 2004), male courtship behaviour in *Drosophila* (Moehring and Mackay

2004). Sucena and Stern (2000), Takahashi et al. (2001) have even shown that trait divergence can be accomplished by mutation at a single gene. But species differences can also be determined by many loci with small effects, e.g. blossom characters in *Mimulus* (Bradshaw et al. 1998, Fenster and Ritland 1994, Fishman et al. 2002) or male genitalia in *Drosophila* (Zeng et al. 2000). Differences in the pheromone blends in noctuid moths (*Spodoptera*) and races of the European corn borer (*Ostrinia nubilalis*) involve mutations in a single gene (Monti et al. 1997, Roelofs et al. 1987), whereas changes in pheromone structure in *Drosophila* are polygenic (Coyne 1996, Coyne and Charlesworth 1997).

As opposed to phenotypic species differences that may act as prezygotic isolation factors, postzygotic isolation involves the interaction of two foreign genomes that never have been jointly exposed to selection. There are two classes of postzygotic isolation: extrinsic and intrinsic isolation. In extrinsic isolation hybrids are fertile vigorous but suffer a fitness-loss compared to the parental species due to intermediate phenotypes that do not match environmental conditions. That is, hybrids may not be competitive in exploiting the ecological niches of the parental species, or they do not fit the signal-receiver system in mate recognition. Evidence for the ecological mode of extrinsic postzygotic isolation came from a hybridisation study between limnetic and benthic morphs of the stickleback *Gasterosteus aculeatus*. Rundle (2002) tested reciprocal backcrosses of the limnetic and benthic morphs and tested them in the natural habitat. Those carrying mostly limnetic genes performed best in the limnetic habitat and vice versa, implying a strong ecological isolation mechanism in that system. Extrinsic hybrid sterility can also be evoked by intermediate behaviour resulting in the rejection by potential mates. For instance, hybrid males of the two tree frogs *Hyla cinerea* and *H. gratiosa*, produce intermediate advertising songs that are unattractive to females of both species (Hobel and Gerhardt 2003).

Intrinsic postzygotic isolation differs profoundly from that of phenotyic species differences because it obligatory involves ubiquitous and strong epistasis[1] between heterospecific alleles in the hybrid genome. Generally, intrinsic postzygotic isolation takes two forms. First, a behavioural dysfunction in hybrids that is effected by neurological or physiological defects that hinder mating efficiency, although fully functional gametes may be produced (see Bock 1984). Second, hybrids may suffer developmental defects causing partial to complete sterility or inviability. Several kinds of genetic irregularities are known to bring about developmental abnormalities: Different ploidy levels of the parental species may disrupt meiotic division, or chromosomal rearrangements lead to unequal recombination. Infections by different cytoplasmic maternally inherited endosymbionts like *Wolbachia* may cause cytoplsamic incompatibilities in many eukaryotes (reviewed in Werren 1997, Stouthmaer et al. 1999, Bordenstein 2003).

Genic hybrid incompatibilities (IC) result from the break up of co-adapted gene complexes and deleterious epistatic interactions evoking hybrid sterility or inviability. It had been shown that genic IC result from inter-locus incompatibilities rather than intra-locus incompatibilities (references in Coyne and Orr 2004, Table 8.2, p. 302-303). Genic incompatibilities arise by alleles that increase fitness or may be neutral in one genetic background but decrease fitness in a recombinant genetic background due to deleterious interactions. Dobzhansky (1934) and Muller (1939, 1940, 1942) proposed the evolution of intrinsic postzygotic incompatibilities via epistatic inter-locus interactions that evolve without an intermediate step opposed by natural selection. The idea of the DOBZHANSKY-MULLER model assumes a species divided into two isolated populations. These two populations start out genetically identical, with an *aa* genotype at one locus and *bb* genotype at another locus. Suppose that in one population the *A* allele gets fixed and the *AAbb*, *Aabb* genotypes are

[1] Interlocus interaction that result in a non-additive effect. Intrinsic postzygotic epistasis leads to a malfunction.

completely fertile and another mutation in the other population gets to the fixation at the other locus with perfectly fertile genotypes *aaBB* and aa*Bb* the intermediate heterozygous step do not suffer a fitness-loss. The crucial point is that both newly arisen mutations A and *B* have not been occurred together in a single genome and the deleterious effect of *A* for instance may appear only when *B* is present and vice versa. Thus, populations can be reproductively isolated by an adaptive valley, although no genotype has crossed the valley.

The model system *Nasonia*

Nasonia are 2-3 mm size long gregarious parasitoid wasps that parasitize fly pupae in bird nest. The genus belongs to the family Pteromalidae within the superfamily Chalcidoidea. The genus consists of the cosmopolitan species *N. vitripennis* and two North-American species *N. longicornis* and *N. giraulti* which may occur syntopically with *N. vitripennis* (DARLING AND Werren 1990). Due to the patchy distribution of hosts, all three species are supposed to have highly subdivided populations and show female-biased sex ratios (Werren 1983, Darling and Werren 1990, Molbo and Parker 1996) as a consequence of inbreeding (local mate competition, Hamilton 1967).

All three species are postzygotically isolated by cytoplasmic *Wolbachia* infections, which cause nucleo-cytoplasmic incompatibilities (Breeuwer and Werren 1990). These incompatibilities lead to a non-recombinant all-male offspring due to the loss of paternal chromosomes (Reed and Werren 1995), since *Nasonia* males are haploid and develop from unfertilised eggs, as in all Hymenoptera.

All three *Nasonia* species are doubly infected with two species-specific *Wolbachia* strains of (Breeuwer et. al. 1992, Werren et al. 1995) that cause bidirectional cytoplasmic incompatibility (IC) (Breeuwer and Werren 1990). Bidrectional IC occurs when male and female harbour different strains of *Wolbachia* that are mutually incompatible (Clancy and Hoffmann 1996). Crosses between *N. vitripennis* and *N. giraulti* (Breeuwer and Werren 1990)

and *N. longicornis* and *N. giraulti* fail to produce viable offspring due to *Wolbachia* induced incompatibilities, whereas in *N. vitripennis* x *N. longicornis* crosses the reproductive isolation is only partial (Werren 1997a).

However, *Nasonia* species can be crossed after elimination of *Wolbachia* induced hybrid incompatibilities by antibiotic treatment yielding viable and fertile hybrids (Breeuwer and Werren 1990). But hybrids suffer incompatibilities of different extent leading to partial embryoinic lethality in males (F_2-hybrid breakdown) (Breeuwer and Werren 1995, Gadau et al. 1999, Bordenstein et al. 2001).

Courtship behaviour

The male courtship display of *N. vitripennis* has been in focus for a long time (Barrass 1960, 1961, 1976, 1979, Assem 1975, 1986, 1996, Jachmann and Assem 1993, 1996). The display consists of well structured motor patterns, so that the entire display can be easily split up in quantifiable courtship components. Thus, the male courtship display represents an excellent model system for the genetic analysis of behaviour.

Nasonia males court on top of the female with the forefeet placed on the female's head. In general, the male courtship display is characterized by periodically repeated series of head nods cum mouthpart extrusions that are separated by pauses of gradually increasing duration (Assem 1975). The number of head nods in consecutive series may vary but show a tendency of increase in the course of a display. There is experimental evidence that the male releases a pheromone every first head nod of a series and that the pheromone plays a key function in evoking receptivity of the female (Assem et al. 1980). Head nodding, mouthpart extrusions and pheromone discharge seem to result from pressure changes in the body. Abdomectomized males court vigorously but without nodding and mouthpart extrusions and hence are incapable of induction of receptivity (Assem et al. 1981). Once a female got receptive it lowers her antennae and raises her abdomen, exposing the genital orfice. The antennal movement at the

onset of the female's receptivity represents the signal for the male to back up in order to get in copulation position. In many chalicidoids males court in a frontal position and are to small to perceive the exposed orfice (Assem and Jachmann 1982). Immediately after copulation, the male gets in courtship position anew and performs a short postcopulatory display, putting the female into an unreceptive condition for the time being (Assem 1986). Virgin females normally get receptive during the first few series but males will show a prolonged display when courting already mated females.

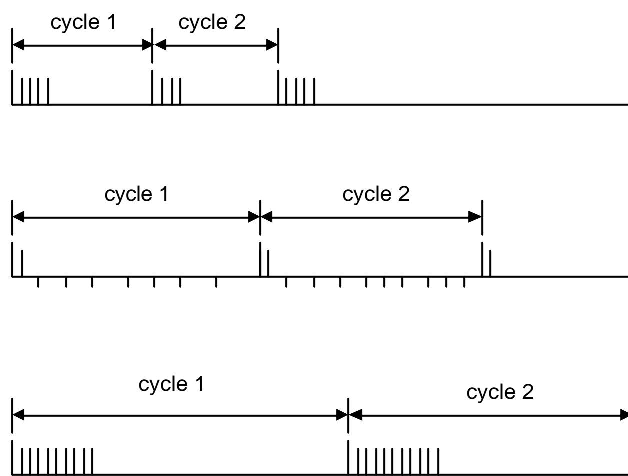

Figure 1: Scheme of courtship patterns in *N. vitripennis* **A**, *N. longicornis* **B**, and *N. giraulti* **C**. Upward bars stand for head nods clustered in series with mouthpart extrusions. Downward bars stand for head nods without mouthpart extrusions.

All three *Nasonia* species differ in the number of head nods per series and in the duration of pauses in between head nod series (Assem and Werren 1994) (Figure 1). *N. longicornis* males show two additional species specific traits: alternated rubbing with the fore tarsi over the females' eyes (**forefeet rubbing**) and irregularly performed nods without mouthpart extrusions in between the head nod series (**minus nods**). Characteristic to the courtship display of *N. vitripennis* is a decrease in the number of head nods in the second series (for details see Assem and Werren 1994, Assem and Beukeboom 2004).

In general, both sexes exchange different modes of stimuli (e.g. visual, tactile, olfactory, acoustical) during courtship, signalling the presence of a mate or advertising positive qualities in order to attain reproductive success (Alexander et al. 1997). Counterintuitivly, the actual courtship display may not primarily play a role in mate recognition or advertisement. Jachmann and Assem (1996) found experimental evidence that the male courtship display in

Nasonia vitripennis mirrored an internal process that cumulatively inhibits the succession of courtship. This idea was already introduced by Barrass (1976, 1979).

The hybrid courtship display is supposed to be intermediate between the two parental phenotypes, but for several courtship components Beukeboom and Assem (2001) found a significant bias towards the paternal line in both reciprocal interspecific crosses[2] between *N. vitripennis* and *N. longicornis*. This behavioural shift has been termed 'grandfather effect' because F_2-hybrid males develop from unfertilised eggs and hence are fatherless but have a grandfather (Beukeoom and Assem 2001, Assem and Beukeboom 2004). Additionally, courtship display of hybrids often exhibit transgressive components, i.e. the phenotype fall beyond the range of both parental strains (Rieseberg 1999, Rieseberg et al. 2000, 2003). For instance, hybrids often show a much higher number of head nod series until giving up and dismounting the female than do the males of the pure lines. It has been suggested that the transgression of phenotypes may be attributed to malfunctioning of endogenous control loops (Jachmann and Assem 1996).

Wing size

Males of all three *Nasonia* species differ in forewing size (Darling and Werren 1990). Males of *Nasonia vitripennis* are incapable of flight due to their vestigial forewings. *N. longicornis* males show an intermeditate wingsize, and males of *N. giraulti* bear fully developped wings similar to the forewings of *Nasonia* females that are capable of flying. Wing size characters in males can even be used as species diagnostic characters (Darling and Werren 1990).

The genetics of wing size differences between *N. vitripennis* and *N. giraulti* comprises major factors that are distributed over all five chromosomes (Weston et al. 1999, Gadau et al. 2002). Additionally, Gadau et al. (2002) detected epistaic interactions that affected wing size

[2] Two crosses between parental strains or species, where the maternal line in one cross represents the paternal line in the other cross.

and seta density on the wings. Although, the adaptive significance of wing size reduction in *N. vitripennis* and *N. longicornis* males have not yet been determined, it has been suggested that wing size reduction may result in faster development to adulthood, or wing size reduction may increase the competitive abilities in male-male competition (Gadau et al. 2002, for other species see also Crnokrak and Roff 1995, Fairbairn and Preziosi 1996, Langelotto et al. 2000). Indeed, *N. vitripennis* males are thought to encounter stronger male-male competition and exhibit a more aggressive behaviour than *N. longicornis* and *N. giraulti* males (Weston et al. 1999). Moreover, *N. giraulti* frequently mates within the host-puparium with high rates of inbreeding leading to a more female biased sex ratio and thus reduced male-male competition (Drapeau and Werren 1999).

Haplodiploidy

The *Nasonia* species complex exhibit several genetic characteristics, which make *Nasonia* a suited system to study the genetics of species differences. In Hymenoptera females are diploid and males are haploid. Dobson and Tanouye (1998) found experimental evidence for a genomic imprinting sex determination (GISD) mechanism in *Nasonia vitripennis*, that has been suggested by and Beukeboom (1995). Accordingly, an unfertilised egg will only bear a maternally imprinted sex determining locus or several loci leading to male development. Whereas a fertilized egg will develop into a female owing to a set of paternally and maternally imprinted sex determining loci. The complementary sex determination mechanism (CSD) in many well studied Hymenopterans obviate the generation and maintenance of specific experimental lines for genetic analyses, e.g. highly inbred lines, recombinant inbred lines (RIL) or nearly isogenic lines (NIL) (for details Lynch and Walsh 1998, pp. 401, 405). Due to the absence of CSD in *Nasonia*, highly inbred lines can be established without generating diploid males (Skinner and Werren 1980) that occur in Hymenoptera with CSD, e.g. in *Apis mellifera* (Beye et al. 2003), *Solenopsis invicta* (Ross et

al. 1993) and *Bombus terrestris* (Duchateau et al. 1994), *Bracon hebetor* (Whiting 1943). In CSD individuals that are heterozygous at the sex determining locus/loci develop into females, whereas individuals that are homozygous (diploid) or hemizygous (haploid) at the sex determination locus / loci develop into males.

Haploidy in hymenopteran males eases the detection of epistatic interaction between incompatible alleles due to the absence of dominance effects that may mask nuclear-nuclear incompatibilities (IC) (Gadau et al. 1999). The absence of dominance effects in haploids also facilitates genome screens for recessive mutations (Pultz et al. 1999, 2000).

Objective

The genetic architecture underlying trait differences is of outstanding interest in evolutionary biology. Formal models have been put forward to provide a theoretical framework of adaptive evolution. Most empirical studies on the genetics of reproductive isolation or species differences are largely confined to a handful of model organisms such as *Drosophila* and *Mimulus* (Coyne and Orr 1998, Orr 2001, Coyne and Orr 2004). However, the understanding of the genetic changes leading to species differences in non-model organisms is still limited.

In order to study the genetic basis of trait differences between closely related taxa, I chose the *Nasonia* species complex because the possibility to generate viable and fertile hybrids between inbred strains represents a powerful method to simultaneously investigate the genetics of phenotyic trait differences and hybrid incompatibilities that are involved in hybrid inviability.

To investigate the genetic architecture of species differences a QTL analysis of the male courtship behaviour and male wing size differences was used.

The genetics of hybrid incompatibilities were investigated with the same genetic framework used in the QTL analysis. Loci that are involved in interlocus hybrid incompatibilities can be

detected through the analyses of allele and haplotype frequencies and can subsequently be mapped onto a linkage map. Therefore, the genetic investigation of phenotyic differences and genetic divergence leading to hybrid incompatibilities can be assessed in a single experimental approach using different statistical analyses.

Material and Methods

Isolation of genomic DNA

DNA was isolated from an entire wasp with a phenol-chlorform procedure. An individual wasp was shock frozen with liquid nitrogen in a 1.5 ml reaction tube and subsequently crushed with a pestle. Then, 100 µl of each DNA-A [10 mM Tris/HCL (pH 7.5), 60 mM NaCL, 10 mM EDTA] and DNA-B [0.2 M Tris/HCl (pH 9.0), 30 mM EDTA (pH 9.0), 2.0 % SDS] buffer were added and the sample was incubated for 2 h with 5 µl proteinase K (10 mg/ml). After the proteinase step, 200 µl phenol were added and the sample was slightly agitated for 10 minutes. Then, the probes were centrifuged for 10 min with 7440 g. the supernatant was afterwards transferred into a new reaction tube. Subsequently, 250 µl chloroform /3-methylbutanol (25:1) were added and the sample thoroughly agitated for 5 sec and then stored at -20°C for 5 minutes. Afterwards, the supernatant was again transferred into a new reaction tube. The DNA was subsequently precipitated with 20 µl sodium acetate (3 M) and 440µl absolute ethanol for 1 hour at -20°C. Subsequently, the sample was centrifuged for 20 minutes with 22780 g. After the centrifugation step the supernatant was discarded and the pellet washed with 100 µl ethanol (70 %). After centrifugation with 22780 g the supernatant was again discarded and the pellet was air dried in a vacuum centrifuge for 10 minutes by 30°C. Finally, the DNA was dissolved in 50 µl low TE (1/10 1 x TE). The DNA samples were stored at -70°C.

Microsatellite development

Genomic microsatellites (simple sequence repeats; SSRs) consist of iterations of 1-6 bp nucleotide motifs that have been considered as evolutionary neutral DNA markers (e.g. Tachida and Iizuka 1992, Awadalla and Ritland 1997, Schlötterer and Wiehe 1999) although their functional signifiance has also been documented (King and Soller 1999, Gur-Arie et al.

2000). SSRs can be used as codominant DNA markers allowing the differentiation of homozgous and heterozygous individuals. The variation is based on size differences detected in gelelectrophoresis.

For the microsatellite isolation an enrichment-procedure was used described in Rütten et al. (2001). Two µg of high molecular weight DNA of *N. vitripennis* was digested with the restriction enzyme *Hinf*I. Adapters (300nM; forward 5'-AXTGGTACGCAGTCTAC-3' where X= Inosine, reverse: 5'-GTAGACTGCGTACC-3') were ligated to the cleavage sites of the DNA-fragments. Enrichment of microsatellite containing fragments was performed by hybridization of a biotinylated $(CA)_{10}$ oligonucleotide subsequent binding of those hybrids to streptavidin coated iron beads and magnetic separation. Five steps of washing with increasing stringency ensured the removement of unhybridized or imperfectly hybridized fragments (Fischer & Bachmann 1998). After denaturation at 95°C for 5 min the remaining fragments were eluted in 50µl dH_2O.

Five µl of the enriched fraction served as template for PCR (polymerase chain reaction) amplification. The PCR reaction was carried out in a total volume of 20µl with final concentrations of 0,2mM of each dNTP, 16mM $(NH_4)2SO_4$, 67mM Tris-HCl (pH8,8 at 25°C), 0,01% Tween, 0,15mM $MgCl_2$ and 0,5U of Taq® DNA polymerase (Biomaster). The PCR reaction was performed in a Trio-Thermoblock (Biometra) with the following profile: 8 min at 94°C, 30 cycles of 94°C for 30 s, 54 °C for 1 min, 72 °C for 1,5 min and a final extension time of 30 min at 72°C.

After amplification 1,5 µl of the PCR-product was directly ligated into pCR®2.1-TOPO® vector (Invitrogen) and finally transformed into One Shot® Chemically Competent *Escherichia coli* cells (Invitrogen). After overnight culture 60 white positive plaques were lifted for plasmid-DNA isolation (Quantum Prep®, Bio-Rad) and sequenced (ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kit).

Table 1
Default setting for primer design in Fast PCR

Tm primer [°C]	50-75
GC content [%]	45-70
Tm of 3' end [°C]	35-45
primr length [bp]	20-30
threshold dimer Tm [°C]	50
CG clamp at 3' end	X

Primer sequences were designed with the program FAST-PCR (www.biocenter.helsinki.fi/bi/bare-1_html/download.htm) with standard settings (Table 1). In some instances, relaxed conditions were used or microsatellites were designed directly from the sequence for the sake of increasing the number of developed microsatellite markers. The optimal annealing temperature $T_M^{opt}[°C]$[3] was calculated according to the formula from Rychlik et al. (1990):

$$T_M^{opt} = 0.7 T_M^{product} + 0.3 T_M^{primer} - 14.9$$

the annealing temperatures were optimized with an Eppendorf Mastercycler® Gradient with a temperature gradient of ± 3°C.

PCR for the amplification of microsatellites were performed in a Eppendorf® Mastercycler in a total reaction volume of 25.0 µl with 1.0 U Taq DNA Polymerase (MBI Fermentas), 1 x PCR buffer with NH_4^+, 2.0 mM MgCl, 0.2 mM dNTPs, 0.4 pmol Primer and 1µl (25 ng) genomic DNA. The PCR-profile comprised 30 cycles at 94°C for 1 min, the appropriate annealing temperature (see appendix 1) for 1 min and 72°C for 1 min after denaturation at 95°C for 10 min and a final elongation step at 72°C for 10 min. For microsatellite analysis 1,8 µl PCR products were run on Spreadex® gels EL 600, EL 500 or EL 300 (Elchrom Scientific). Gels were stained with SYBR™ Green I (0.01 %) for 25 minutes in distilled water, and destained for one hour. Gels were documented with the gel imaging system DOC-print (ltf Labortechnik Gmbh & Co KG, Wasserburg). PCR products of the isofemale inbred lines ASYMC and IV7R2 served as size standard.

[3] T_M : melting temperature

AFLP marker (amplified fragment length polymorphism)

AFLPs are fragments obtained from endonuclease restriction, followed by ligation of oligonucleotide adapters to the fragments and subsequent selective amplification with PCR-primers that consist of a core sequence, a restriction-enzyme specific sequence and one to three selective nucleotides (Vos et al. 1995). AFLPs are dominant DNA markers, i.e. the detectable polymorphisms are only scored as present or absent so that heterozygous individuals can not be distinguished from homozygous individuals.

The genomic DNA was double-digested with the rare cutter *EcoRI* and the frequent cutter *MseI* restriction enzymes. The DNA fragments were T4 ligated with *EcoRI* and *MseI* adaptors, generating template DNA for a pre-selective PCR amplification *EcoRI* and *MseI* primers with an additional nucleotide[4]. In a second selective PCR a labeled *EcoRI* primer and an *MseI* primer were used with three additional nucleotides at the 3' end allowing for each primer combination to amplify different subsets of fragments.

PCR products were visualized with an ABI 377 automated DNA analyzer (Applied Biosystems) after labeling the *EcoRI* primers with a fluorescent dye (FAM, HEX or NED). Fragment sizes were determined using GeneScan computer software provided by the manufacturer and GenoGrapher 1.6.0 software (Copyright 1999 Montana State University-ware). Primer sequences for the pre-selective and selective PCR and labeling are shown in appendix 2.

[4] Selective nucleotides are indicated in the primer name by +x, e.g. EcoRI+xxx designates a primer that is specific to the EcoRI adaptor sequence with additional three nucleotides that are unspecific.

AFLP protocol (for ten samples)

Used chemicals

MseI (10 U/µl) (New England Biolabs)
EcoRI (10 U/µl) (New England Biolabs)
T4-DNA ligase (5 U/µl) (Roche)
Taq-polymerase (5 U/µl) (Roche)
AFLP amplification core mixture (Applied Biosystems Inc.)
Genescan Rox 500 internal lane standard (Applied Biosystems Inc.)

Preparation of adaptor solution

1 µl EcoRI-ad1 (10µM)	CTCGTAGACTGCGTACC
1 µl EcoR1-ad2 (10µM)	AATTGGTACGCAGTCTAC
9 µl MseI-ad1 (10µM)	GACGATGAGTCCTGAG
9 µl MseI-ad1 (10µM)	TACTCAGGACTCAT

Incubate for 10 min by 65 °C

Digestion and ligation with EcoRI and MseI and T4 DNA ligase

3.0 µl	*MseI*
0.6 µl	*EcoRI*
17.0 µl	Eco-Mse adapter
1.0 µl	T4 DNA ligase
27.5 µl	T4 DNA ligase buffer (10X)
170 µl	MilliQ sterile water

Add 20 µl reactionmix to each tube or plate-well with 5 µl DNA (25ng/µl), spin for 10 sec and incubate the tubes or 96-well plate for 2 h at 37 °C. Then heat the samples 10 min at 65 °C. Cool down to 20 °C

Pre-selective PCR of restriction-ligation mix

125.0 µl	AFLP core mix
1.5 µl	EcoRI+x primer
13.0 µl	MseI+x primer
6.0 µl	MilliQ sterile water

Add 1.5 µl of the digestion-ligation mix to 13 µl of pre-selective PCR mix in the 96-well plate, spin for 10 sec before PCR.

PCR program

1) 72 °C	120 sec
2) 94 °C	5 sec
3) 56 °C	30 sec
4) 72 °C	120 sec
5) goto 2)	19 x
6) 4 °C end	

Selective PCR amplification

Dilute the PCR product from the pre-selective amplification 4-20 times with MilliQ, depending on amount and quality of the amplicon.

Mastermix

11.0 µl	10 x PCR mix
11.0 µl	2mM dNTP
11.0 µl	10mM MgCl2
1.0 µl	Taq
5.0 µl	Labeled EcoRI+xxx primer
1.5 µl	MseI+Cxx primer
60.0 µl	Milli Q sterile water

Pipette 9 µl of the master mix in each well of the 96-well-plate. Add 1 µl of the diluted pre-amp to each well and spin the 96-well plate for 10 sec.

PCR program

1) 94 °C 5 sec
2) 65 °C 30 sec
3) 72 °C 120 sec
4) goto 1 for 8 times and with temperature increment of 1 °C per cycle
5) 94 °C 5 sec
6) 56 °C 30 sec
7) 72 °C 120 sec
8) goto 5 for 24 times
9) 72 °C 10 min
10) 4 °C end

Sample preparation for gel electrophoresis

Prepare a mix of
0.8 µl AFLP-product (FAM)
0.8 µl AFLP-product (NED or HEX)
1.3 µl loading mix with GT500 (ABI)

Denaturate 2 min on 94 °C, than store on ice. Load 1.3 µl on the gel.

RAPD (random amplified polymorphic DNA)

RAPD primers are generated with a PCR of a single 10mer oligonucleotide, which binds to many different loci generating random amplified sequences of different size. The PCR reaction only yields an amplified fragment when the short oligonucleotide binds to both complementary DNA strands in opposite direction in a distance that is amplifiable in PCR. PCR-products were separated on 20 x 25 cm horizontal gels using 1 % Synergel (Diversified Biotech, Newton Center, MA) and 0.6 % Agarose in 0.5 x TBE buffer (Sambrook et al. 1989). Gels were run for 500 Vh, and subsequently stained with ethidium bromide (0.01 %) for 25 minutes and destained in distilled water for 40 minutes. Gels were documented with the gel-imaging system DOC-print (ltf Labortechnik GmbH & Co KG, Wasserburg, Germany). The primers A20, C16, E01, X04 and Y07 were selected from the Roth random primer kit (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) for RAPD PCR.

The PCR was performed on a Biometra® T1 theromocycler in a total reaction volume of 12.5 µl with 0.25 U Taq DNA Polymerase (MBI Fermentas), 1 x PCR buffer with NH_4^+ , 2.0 mM MgCl, 0.1 mM dNTPs, 0.6 µmol primer and 1µl (5ng) fivefold diluted genomic DNA. The PCR-profile comprised 5 cycles at 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min, respectively and another 32 cycles at 94°C for 10 sec, 35°C for 30 sec, and 72°C for 30 sec, respectively.

Isolation of chromosome specific markers

In order to develop chromosome specific markers that can be used as anchor points for physical mapping markers were developed from single microdissected metaphase plate chromosomes. The haploid chromosome set in *Nasonia* is composed of five metacentric chromosomes gradually decreasing in size (Pennypacker 1958, Gokhman and Westendorff 2000). Chromosomes of *N. vitripennis* (ASYMC) were prepared from prepupal cerebral ganglia (see Imai et al. 1988 for chromosome preparation in ants).

The prepupal stage could easily be recognized ((c) in Figure 2) and were collected at about the eighth day after oviposition at 25 °C. Due to the minute amount of tissue, a combined technique of the protocol of Imai (copy circulated by the author) and a 'squash technique' was applied to reduce the loss of tissue (see also Hillis 1996, protocol 4 p. 150). The chromosome preparation was made on 60 x 12 mm coverslips for the subsequent microdissection procedure under an inverted microscope.

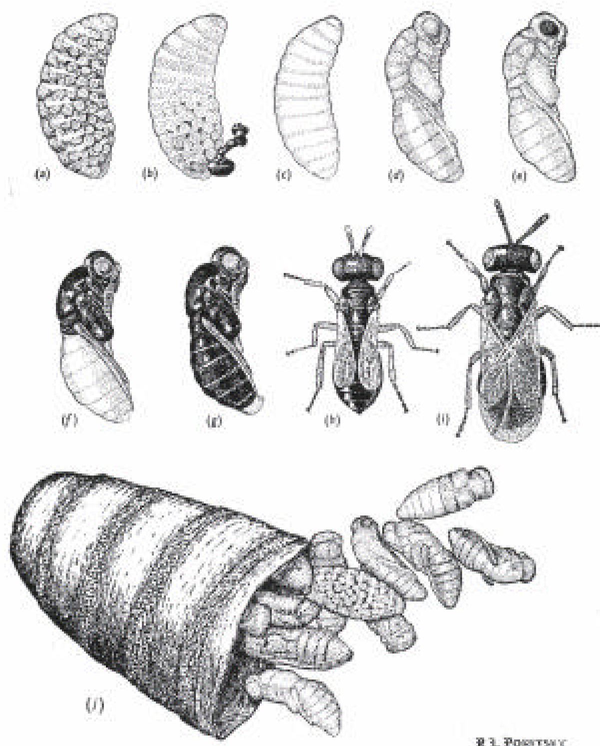

Figure 2: Developmental stages of *N. vitripennis*. (a) Diapausing larva, (b) defaecating larva, (c) early prepua, (d) pink eyed pupa, (e) red eyed pupa, (f) pupa with black head and thorax, (g) completely sclerotised pupa, (h) adult male, (i) adult female, (j) Sarcophaga puparium broken open to reveal enclosed diapausing larvae and pupae.
(Figure from Schneiderman and Horwitz (1958)

The tissue was incubated in hypotonic colchicin solution (0.005%) for one hour. Thereafter, the tissue was transferred with a pipette on a ethanol cleaned cover slip. Excess of hypotonic solution was soaked up with a pipette and the tissue was rinsed on the inclined cover slip with droplets of 60 % acetic acid - ethanol fixative. Then the tissue was macerated in a few droplets of 60 % acetic acid -ethanol

fixative for one minute and the fat body and tracheae were removed with dissecting needles. The macerated tissue was minced with dissection needles until a cloudy cell suspension was obtained. After slight evaporation of the fixative, the cell suspension was surrounded by droplets of absolute glacial acetic acid - ethanol fixative to prevent the loss of tissue when a droplet of fixative was directly applied on the sample. An 8 x 8 mm siliconized cover slip was subsequently laid upon and than pressed thoroughly with the thumb. A paper towel was laid upon of the cover slip to absorb an excess of fixative. The probe was finally fixed in Carnoy's fixative (ethanol (100%): glacial acetic acid 3:1) for 15 minutes and than air dried.

The chromosome microdissection and marker development was mainly carried out by K. Rütten in colaboration and was recently published (for details see Rütten et al. 2004). For this reason, only a comprehensive description is given.

For differential diagnostic air dried chromosomes were GTG-banded. The microdissection was carried out using fine glass needles attached to an Eppendorf micromanipulator and an inverted microscope (Zeiss Axiovert 2000) with 1000 x magnification. Before the microdissection step a drop of water was placed on the sample to avoid the loss of chromosomes through electrostatics during microdissection. The chromosome was subsequently transferred into a collection buffer containing proteinase K (purified for PCR), DOP-PCR primer and dNTP of each type. After incubation at 37°C overnight a preamplification of eight cycles with T7 DNA polymerase was performed. Following this preamplification step 35 cycles of a conventional PCR with DOP-PCR primers were conducted. To verify the success of the microdissection and to estimate, and the degree of contamination of PCR products, a dot blot experiment was conducted. The purity and quantity of the DOP-PCR amplified DNA was assessed with anti-Dig-Fluoresence Fab-fragments (Roche) and CDP-Star (Roche). The generation of the probe was conducted via a second DOP-PCR with digoxygenin-11-dUTP. The enrichment procedure of repetitive genetic elements was similar to that used for the microsatellite marker development (see

above). Chromosome specificity of the PCR products were tested with a sequential fluorescence insitu hybridisation (FISH) experiment.

Microdissected markers were amplified in a LV[5] hybrid mapping population of 94 males and mapped with microsatellite markers and RAPD markers of E01, X04, X08, Y13, Z08, Z20 from the Roth random primer kit (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) (Linkage analysis see p. 25).

Nasonia breeding and establishment of a F_2 hybrid mapping population

Nasonia stocks were bred at 25°C under 16 h light and 8 h darkness. The relative humidity amounts to 50 - 60 percent. The life cycle under lab conditions took 14 days (for details Schneiderman and Horwitz 1958, Whiting 1967, Pultz and Leaf 2003). *Nasonia* cultures were maintained on *Calliphora spec.* fly pupae, purchased commercially as larvae. After pupation the fly pupae were incubated at room temperature for 3 days and subsequently stored at 4°C maximum of one month. Fly pupae that are appropriate for parasitation have already passed the larval moult but do not show any eye pigmentation.

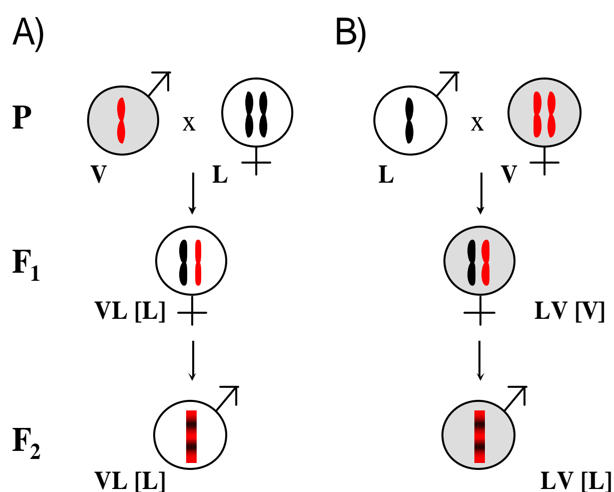

Figure 3: Breeding scheme of eciprocal interspecific crosses between *N. vitripennis* (**V**) and *N. longicornis* (**L**). In the denotation of hybrids the paternal genotype is followed by the maternal genotype and the cytotype is indicated in brackets. **A)** VL[L] crosswith the *N. longicornis* cytotype. **B)** LV[V] cross with a *N. vitripennis* cytotype.

[5] L stands for the *N. longicornis* genotype and V for the *N. vitripennis* genotype. Hybrids are designated by the paternal genotype followed by the matgernal genotype.

For the production of hybrid males the *Wolbachia* cured inbred lines ASYMCHS (*N. vitripennis*) and IV7R2 (*N. longicornis*) were used to yield hybrid offspring (Figure 3). For the establishment of interspecific hybrid populations, virgin females were collected in the pupal stage to prevent conspecific mating. After emergence, the females were mated with heterospecific males. Thereof, F_1-hybrid females were selected one to two days prior to eclosion and set as virgins on fly pupae to produce a recombinant all male offspring designated as LV[V] hybrids in one cross and VL[L] hybrids in the reciprocal cross. L stands for *N. longicornis* and V for *N. vitripennis* genome, the mitotype is put in parentheses. In the following the hybrid populations are designated with LV or VL.

Phenotyping of F_2-hybrids and parental strains

The male courtship was split up into distinct components (Figure 4). A total of 480 hybrid individuals and 93 of pure ASYMC and 72 IV7R2 parental strains were phenotyped. The interval between the 1st head nods of two consecutive series is termed a cycle. The

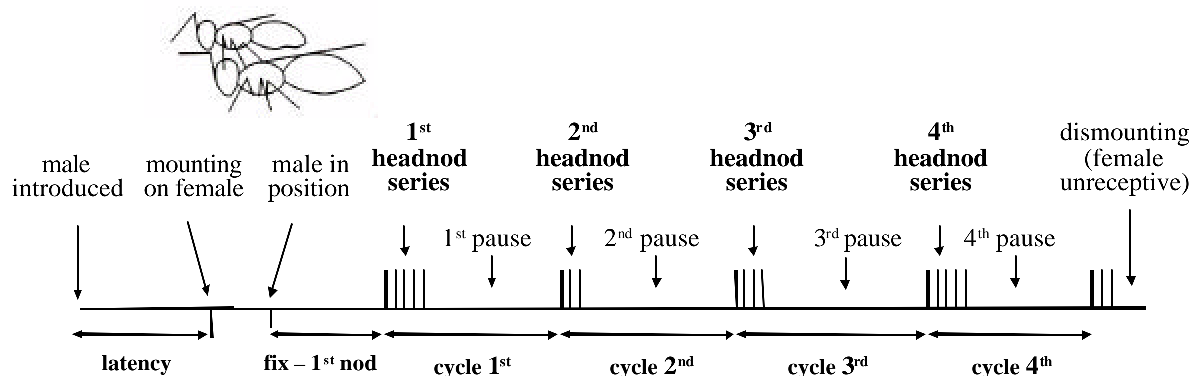

Figure 4: Scheme of the male courtship display in *N. vitripennis*. Vertical bars represent separate head nods. First nods (marked in bold face) coincide with the release of male pheromones that provoke receptivity. Recorded courtship components are marked in bold face.

number of head nods (**hnd**) and **cycle** time in four consecutive cycles were recorded. Additionally, the time of the males' rapprochement toward the female called **latency**, and a courtship component called **fix-1st nod**, which is the time after the female got immobilized by the male and the onset of the courtship display were recorded.

N. longicornis males show two additional species specific traits: alternated rubbing with the fore tarsi over the females' eyes (forefeet rubbing) and irregularly performed nods without mouthpart extrusions in between the head nod series (minus nods). Characteristic to the courtship display of *N. vitripennis* is a decrease in the number of head nods in the second series, termed h2-h1 (Assem and Werren 1994). Observations were made with 12 x magnification under a dissection microscope. Polystyrol tubes (75 mm x 12 mm ∅) served as observation chambers.

F_2 LV males and ASYMCHS test-females were of controlled age (2-3 days post-emergence). Males were inexperienced, whereas females were already mated to prevent premature termination of courtship due to copulation. We used the software package THE OBSERVER 2.0 (Noldus Information Technology, Wageningen, Netherlands) for the recording of the courtship display. Observations were ceased and the male discarded, if a male did not mount on the female within 5 min.

Forewings of 94 LV-hybrid males that have courted were mounted on slides and embedded in Entellan (Merck, Darmstadt). All measurements were carried out with 40 times magnification. Wing length was measured from the distal end of the wing to the mass of dark connective tissue at the proximal end. Wing width was measured perpendicular to the wing length axis. Head width was measured as the inter-ocular distance at the level of the first ocellus. This measure is correlated with relative body size in *Nasonia* (Skinner 1983) and has been used to normalize wing-size measurements and remove the effects of body size. Wing setae in *Nasonia* are fine hairs on the upper and lower side of the wing. Seta density was measured within a 0.0156 mm^2 grid that was located with the upper proximal edge at the tip of the stigmal vein according to Weston et al. (1999) and Gadau et al. (2002). Besides the basic measures, a composite measure of wing length x wing width that was corrected for body size by dividing by the inter-ocular distance was used (Weston et al. 1999).

Additionally, fifteen individuals of both parental strains were phenotyped for wing-size measurements.

Phenotyping the courtship behaviour of wildstrains

Nasonia vitripennis wildstrains were collected from a field population in Schlüchtern (Germany) in order to estimate the phenotypic variation occuring in natural populations. *Nasonia* wasps were baited with fly pupae that were deposited in little gauze bags in nestboxes after the breeding season. One week later the fly pupae were separated in polystyrol tubes and cultured for seven days at 25 °C. Then virgin females were collected in the pupal stage and cultured until emergence. Seventeen virgin females from different nestboxes were collected and set on fly pupae to yield an all-male offspring that had been scored for courtship behaviour. Wildtype males were tested on mated females of the *N. vitripennis* inbred strain ASYMC. A total of 190 males had been scored (see appendix 12).

Phenotype statistics

The phenotypic distributions for continuous traits were tested for normality or a log-normal distribution with a Kolmogorov Smirnov test and discrete traits were tested against a Poisson distribution with a χ^2-test for the goodness of fit. Phenotypic coupling for traits measured on the same scale were inferred with the rank correlation coefficient (Spearman's ?) or with a χ^2-test for binary traits. Statistical association between of the number of head nods and continuous traits (cycle time, latency, fix-1st nod) were tested with a Mann-Whitney *U*-test between two head nod classes according to whether they were *N. vitripennis* or *N. longicornis*-like because there is no overlap in the phenotypic distributions between the parental strains. Two head nods were used as the upper limit to allocate a hybrid into the *N. longicornis* class and four head nods as the lower limit to assign an individual to the *N. vitripennis* class (see Beukeboom and Assem 2001). Before applying a U-test a rank dispersion test (Mood 1954) was carried out to test for homogeneity of variances. The trend in

the number of head nods and cycle time in the four cycles were tested with Kendall's coefficient of concordance. Owing to the large sample size the coefficient of concordance were transformed into Fisher z to test for significance (Kendall and Gibbons 1990, pp. 121-122).

To control the type I error for multiple tests the significance threshold was adjusted according to the Dunn-Šidák procedure ($a' = 1-(1- a)^{1/k}$, where k = number of markers and a = 0.05, Sokal & Rohlf 1995, p. 239). Standard statistics were calculated with the software Statistica 6.1 and SPSS 11.5.

Linkage analysis

The construction of a linkage map represents a statistical procedure yielding a most likely order of genetic markers estimated on the basis of recombination frequencies between loci. Recombination frequencies are translated in map distance, normally expressed in centi-morgan [cM]. A map distance of 1 cM corresponds to one recombination event in 100 meioses. The relationship between recombination frequency and genetic distance is mediated by different mapping functions that account for different degrees of interference in crossing-over. A crossing-over event exerts interference when it reduces the probability of crossing-over events at adjacent loci than expected under complete independency between loci.

The linkage map was generated with the software package JoinMap 3.0 (JM) (STAM 1993). The mapping procedure in JM can be split up in the following basic steps. First, this program calculates the LOD score of linkage between pairs of loci. The LOD score is a logarithimic measure of the ratio of the likelihood that two loci are linked over the likelihood that these loci are unlinked. In a second step, linkage pairs of loci are grouped on the basis of LOD scores and recombination fractions, beginning with the pair of loci with the highest LOD score. In consecutive steps markers are determined, which are to be added to the linkage

group until a user-supplied critical LOD value is reached. In all experiments a critical LOD value of 5.0 and a threshold of ? = 0.35 recombination fraction for marker grouping was used. Map distances were calculated with the Kosambi map function. Since the estimated genetic distances are not equally accurate, putative discrepancies of inferred genetic distances between a two-point versus a three point estimate is weighted by JM using a least square procedure (Stam 1993). After assigning weights to the final pair-wise data, a numerical search for the best fitting for linear order is performed. Every time a new marker is added to the existing map, JM permutates the existing locus order in a three marker window throughout the whole linkage group to find the most likely order. In the final step, weighted genetic distances between pairs of loci in a linkage group are translated in a genetic distance estimate comprising the whole linkage group.

A total of four maps were constructed for genetic analysis: a LV map was based on 94 individuals, a VL map based on 82 individuals and an AFLP map based on a mapping population of 326 LV individuals were calculated. Finally, a map comprising of shared markers of the first LV and VL mapping population was calculated as a reference map (LV_VL map) for comparative analysis of marker statistics in reciprocal crosses. Before the genotype data were pooled locus order and multipoint map distances are tested for homogeneity between the two mapping populations with a likelihood ratio test implemented in the PGRI software (Liu 1998). This application was only restricted to loci that were in common in both reciprocal crosses.

In order to assess the confidence of locus order and the impact of a marker on the locus order, a bootstrap procedure and a combination of bootstrapping and jackknifing with hundred replicates was applied to the locus order of the LV_VL map to obtain an estimate of the percentage of correct locus ordering (PCO) (Liu 1998, pp.291-299). For k loci the PCO is calculated according to the formula (1), where Pii is an indicative for the obtained probability of the locus position in the bootstrap procedure.

$$(1) \qquad PCO = \frac{1}{k} \sum Pii$$

With the bootstrap-jackknife approach the impact of a single locus on the overall confidence of a locus order is estimated by performing a bootstrapping procedure after discarding a locus from the analysis. If the PCO of the estimated gene order decreases significantly after a locus has been discarded, the locus will be excluded from further analyses.

Experimental design of QTL analysis

In the QTL analysis of the male courtship behaviour in the LV[6] cross an experimental design was applied that started with a mapping population of moderate size followed by an independent analysis of an equally sized mapping population in order to confirm previously detected QTL. Finally the sample size was increased threefold in order to reduce the sampling error of QTL effect estimation. For the first analysis a subset of 94 LV hybbrid individuals were selected from the lowermost and uppermost fraction of 480 phenotyped individuals for either trait. Known as selective genotyping, this strategy can result in an increased power to detect a QTL due to an increase in phenotypic mean differences between the marker classes (Lander and Botstein 1989, Carey and Williamson 1991, Darvasi and Soller 1992). However, the strength of the selection procedure was reduced since multiple traits were considered, i.e. a higher trait value in one courtship component is not necessarily be associated with a high value in another trait.

QTL effects are notoriously overestimated in small mapping populations (Beavis 1994, 1998). Given a normal distribution of error about a true QTL effect, errors involving overestimation surpass the significance threshold more often than underestimated errors. The standard error (SE.) of QTL effect estimation can be reduced by increasing sample size

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[6] L stands for the *N. longicornis* genotype and V for the *N. vitripennis* genotype. Hybrids are designated by the paternal genotype followed by the maternal genotype.

according to the equation $SE. = \frac{s}{\sqrt{n}}$. To investigate the impact of sampling error on QTL effect estimation a mapping population of 298 individuals were genotyped with AFLP marker. From that pool random samp les were chosen of 95, 147, 196, 257 and 298 genotyped individuals[7].

MapQTL® 4.0 (DLO Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands) was used for the interval mapping (Lander and Botstein 1989). When the QTL location could not be resolved due to broad LOD score peaks over the linkage groups, a MQM procedure as implemented in MapQTL® 4.0 was applied. In a forward approach cofactors were selected to control for genetic background variation. The best resolution was achieved by using the marker with the highest LOD score as a cofactor. With that approach the genetic variation of that marker (proxy for a real QTL) can be fitted to reduce the phenotypic variance and to increase the power to detect additional QTL on the linkage group.

Binary traits as the presence or absence of forefeet rubbing or minus nods were mapped using a Fisher-exact test for two locus linkage disequilibirum (LD) by coding these binary traits as biallelic markers. The analysis was carried out in GENPOP ON THE WEB (http://wbiomed.curtin.edu.au/genepop/). In the QTL analyses for courtship behaviour, the total number of head nod series were transformed in a binary trait according to whether an individual showed transgressive (> 20 series) or normal behavior and this trait was also mapped via LD.

A genome wide significance threshold for QTL detection was obtained with a permutation test (except for binary traits) implemented in MapQTL 4.0, yielding a LOD score distribution under the null-hypothesis (Churchill and Doerge 1994).

[7] The sample size for QTL analysis may deviate from the number of randomly drawn samples due to missing phenotypes.

Genome Interactions

The software package Epistat (CHASE ET AL. 1997) was used to screen the whole genome for two-way non-additive interactions between QTL in the QTL analysis based on 94 LV-hybrid males. This program detects epistatic interactions among primary QTL (detected already in interval mapping) and conditional QTL (secondary QTL) that show a phenotypic effect only in conjunction with a primary QTL (CHASE et al 1997). The alleged standard settings in Epistat were used (see Manual http://64.226.94.9/epistat.htm). The inferred LOD scores for the presence of an epistatic interaction were transformed in p-values generated with a Monte-Carlo simulation implemented in Epistat (Chase et al. 1997). The p-value was adjusted to account for the search over 5 chromosomes (GADAU ET AL. 2002, LARK ET AL. 1995) with the Dunn-Šidák correction.

Evidence for genome interactions that are involved in hybrid incompatibilities come from two sources: marker frequencies and association. Nuclear-cytoplasmic incompatibilities (nuc-cyt IC) in the reciprocal crosses LV and VL were investigated on a global scale testing the mean recovery of N. vitripennis alleles of mapped markers of a mapping population a with a t-test with a directed nullhypothesis of a 1:1 ratio of parental alleles. The distribution of marker recovery was tested against a normal distribution with a Shapiro-Wilk test. Additionally, deviations from an expected 1:1 ratio per mapped marker were tested with a $?^{2}$-test for the goodness of fit on a locus specific scale (Yates corrected; Fowler and Cohen 1996, p. 119). The distribution of loci that showed segregation distortion was visualise with a composite graphical genotype on LV_VL map for both reciprocal crosses to detect differences and similarities in the segregation ratios in both reciprocal crosses (see Rieseberg et al. 1996). In the composite graphical genotype the frequency of *N. vitripennis* alleles were displayed in three categories (significant deficiency of *N. vitripennis* alleles, 1:1 ratio of parental alleles, and significant excess of *N. vitripennis* alleles).

Hybrid incompatibilities between different chromosomes were tested with two-locus linkage disequilibirum (LD) with a Fisher-exact test in GENPOP ON THE WEB (http://wbiomed.curtin.edu.au/genepop/). A Dunn-Šidák correction was used for a adjustment over the number of significant LD on the 0.05 level.

The measure of linkage disequilibrium tests for non-random association of haplotypes in a two-locus system and can be used to detect incompatibilities of alleles in a hybrid cross comprising nuclear-nuclear incompatibilities (nuc-nuc IC), nuclear-cytoplasmic incompatibilities (nuc-cyt IC), i.e. the negative interaction with nuclear and cytoplasmic factors.

In a more detailed analysis markers that occurred in both reciprocal crosses and that had been mapped on the LV_VL map were analysed, to compare the distribution of hybrid incompatibilities in the genome of the recirpocal LV and VL crosses. For that analysis only marker pairs were used, that showed significant LD in both reciprocal crosses. The influence of the cytoplasm on nuclear genes was inferred with a ?^{2}-test for the goodness of fit of a 1:1 ratio of parental alleles (**A**+**C** versus **B**+**D**; **A**+**B** versus **C**+**D**) (Table 2). Nuclear-nuclear incompatibilities were detected through a significant excess of conspecific haplotypes (**A**+**D**) versus the heterospecific haplotype frequencies (**B**+**C**). With these tests, significant LD that resulted from hybrid incompatibilities could be separated into nuc-cyt IC, nuc-nuc IC and in coincidence of both IC types nuc-nuc-cyt IC. The combined effect of nuc-nuc and nuc-cyt IC may lead to an excess of the maternal haplotype frequency, that have been tested with a ?^{2}-test between the frequency of maternal and paternal haplotypes (**A** versus **D**) Dunn-Šidák correction was used for a adjustment over the number of marker combinations that show significant LD on the 0.05 level in both reciprocal crosses.

Table 2

Contingency tables for combinations of *N. vitripennis* and *N. longicornis* genotypes between two alleles of two loci in a hybrid mapping population. 1) scheme 2) example of hybrid haplotype deficiency.

1)

marker B \ marker A	V	L
V	conspec. **A**	hybrid **B**
L	hybrid **C**	conspec **D**

2)

Nv-39 \ Nv-46	V	L	
V	40	11	51
L	13	26	39
	53	37	90

V: *N. vitripennis* allele, L: *N. longicornis* allele; Nv-39, Nv-46: loci for which significant linkage disequilibrium has been detected; conspec.: conspecific haplotype (VV or LL), hybrid: heterospecific haplotype (LV or VL).

Results

Marker systems

Microsatellite marker development

A total of 60 inserts of positive clones were sequenced and 49 of them contained dinucleotide repeats. In 20 sequences the microsatellites were composed of perfect repeats. 22 microsatellites showed interrupted repeat sequences and four of them were composed of two dinucleotide repeat types. Four composed microsatellites show no interruption between the different nucleotide repeats (see appendix 1). For 49 microsatellites primers were developed and 35 could be cross-amplified in *N. longicornis*.

AFLP

Combinatins of selective primer yielded yielded up to 24 mapped markers (Table 3). The number of genotyped markers differed between between the mapping populations LV (n = 94), VL (n = 82) nd LV (n = 326). The highest proportion of mapped markers was achieved in the LV population based on 94 individuals. However, this might be affected by the lower number of genotyped markers per selective primer combination.

Table 3

AFLP loci of selective primer combinations in three different mapping populations (for primer notation see appendix 2).

primer combination	LV (n =94) map.*	LV unmap.*[1]	primer coding	VL (n = 82) map.	VL unmap.	primer coding	LV (n = 326) map.	LV unmap.
AB	5	6	BC	13	9	CE	22	23
AC	0	8	CE	14	32	CG	6	32
AD	7	0	CG	2	37	EB	8	33
BC	4	0	EB	24	21	EC	3	29
CD	3	6	EC	1	36	FA	15	21
CE	3	3	FA	15	22			
CG	6	1						
DD	1	8						
EB	5	4						
EC	4	1						
ED	1	7						
FA	6	0						
FB	2	8						
FD	2	8						
HC	1	1						
S	50	61		69	157		54	138

*: markers that have been mapped on a linkage group.
*[1]: markers that could not be assigned to a linkage group

Chromosomal anchoring

Chromosomal metaphase plates appropriate for chromosome microdissection have been detected in 90 percent of all cover slips. Only methaphase plates were used, when the chromosomes were sufficiently separated from each other (Figure 5). The number of metaphase plates per cover slip ranged from 1 to 28. Chromosomes were differentiated according to size and GTG banding pattern (Figure 6 a). The chromosome specificity was finally tested in a multi-color fluorescence in situ hybridisation (FISH) experiment with DOP-PCR products of all five chromosomes (Figure 6 b). The FISH experiments were carried out by Michaela Neusser (department of human genetics and anthropology, LMU Munich).

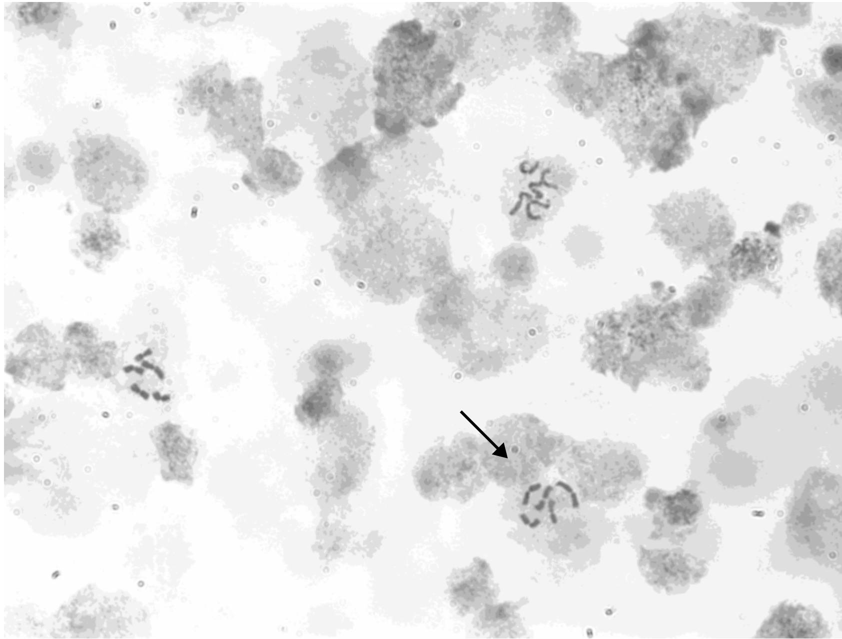

Figure 5: Metaphase plates from cerebral ganglion tissue of a male prepupae (haploid n = 5, Giemsa stained, 400 x magnification). The metaphase plate appropriate to chromosome microdissection is marked with an arrow.

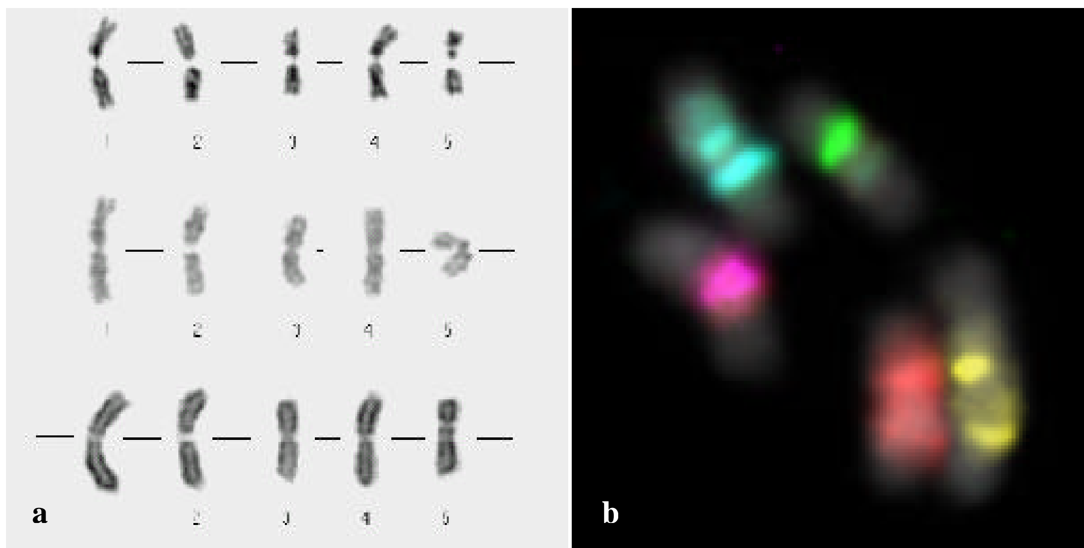

Figure 6: (**a**) GTG-banded chromosomes of three different *N. vitripennis* males. Chromosomes are numbered and ordered according to size and structure (see Gokhman and Westendorff 2000). (**b**) Multicolor FISH with chromsome-specific DOP-PCR products as probes (chromosome 1 = yellow, chr. 2 = purple, chr. 3 = red, chr. 4 = light blue, chr. 5 = green).

After the development of chromosome specific markers by Karsten Rütten, 94 LV hybrid males were genotyped for chromosome specific markers, RAPD and microsatellite markers. 47 out of 54 markers could be mapped to five linkage groups with grouping and mapping thresholds of LOD 3.0 and recombination fraction of 0.35. In that mapping experiment the microsatellite and RAPD markers that have been developed from nuclear DNA extraction

could be assigned to specific chromosomes (anchor map) (Figure 7). Additionally, linkage groups of a RAPD map were homologised through shared markers with the anchor map. From the anchor map and the homologised RAPD map markers can be used to homologise and assign linkage groups to specific chromosomes in mapping experiments of crosses comprising either the *N. vitripennis* ASYMC strain or the *N. longicornis* IV7R2 strain.

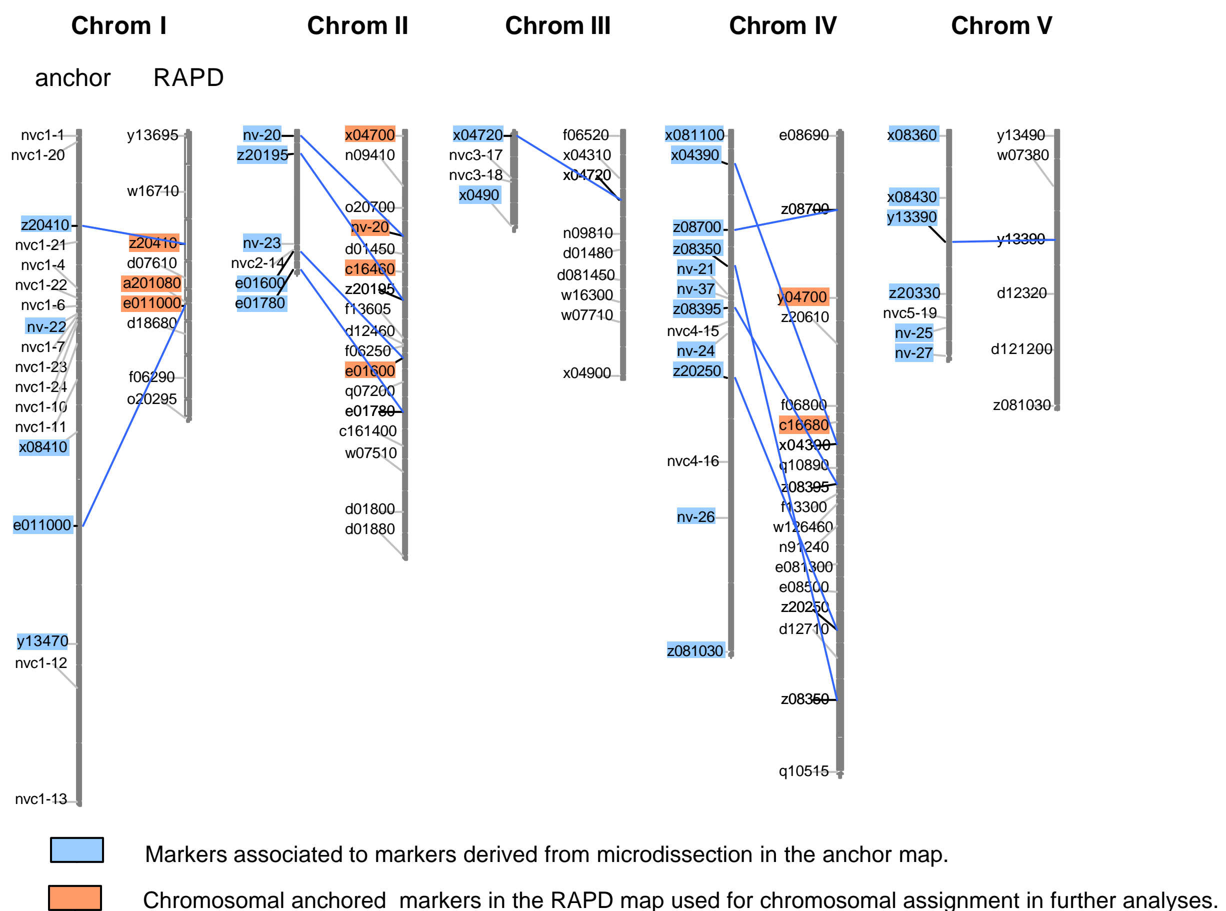

Figure 7: Chromosomal anchored linkage maps based on two *N. longicornis* (?) x *N. vitripennis* (?) LV mapping populations. The linkage groups on the left side (anchor map) are based on STS markers derived from chromosome microdissection, and RAPD and microsatellite markers derived from whole genome extractions. Linkage groups on the right side are predominantly based on RAPD markers and were chromosomal anchored by shared RAPD or microsatellite markers with the anchor map indicated by dashed lines.

Linkage map construction

Linkage data of three different mapping populations are presented: 1) a *N. longicornis* (?) x *N. vitripennis* (?) cross (**LV**), 2) a *N. vtiripennis* (?) x *N. longicornis* (?) cross (**VL**) and 3) an intraspecific *N. vitripennis* cross between the two field lines R moscow (Russia) and a HV isofemale line (Hoge Veluve, the Netherlands) (**intraspecific**) (Table 4). The grouping

of two-point linkage between loci and the mapping were performed with different LOD score thresholds among the different mapping populations to yield a set of five linkage groups. Only the mapping threshold was kept constant by 0.35 recombination frequency. The RAPD markers, included in the LV map, had been selected from a RAPD linkage map used in a pilot QTL analysis on the male courtship behaviour in a *N. vitripennis* x *N. lonigcornis* cross (Gadau unpublished). The microsatellite markers showed the highest mapping efficiency in all three mapping experiments with 50 - 90 % mapped markers, whereas the mapping efficiency of AFLP markers ranged from 45 to 26 % (Table 5). The map length between the different mapping populations differed up to a factor of 1.6 between the LV and the intrapsecific map (statistics see Table 4). Recombination fractions of the interspecific crosses were tested for shared micosatellite markers in respect to the recombination fraction of the intraspecific cross (Figure 8). Recombination fractions in the VL cross did not differ significantly from intraspecific recombination fractions, whereas they differed significantly between the LV cross and the intraspecific cross (*Wald-Wolfowitz runs test*: **LV**: $z = -2.157$, $p = 0.03$, $n = 28$; **VL**: $z = 0.00$, $p = 1.0$, $n = 28$).

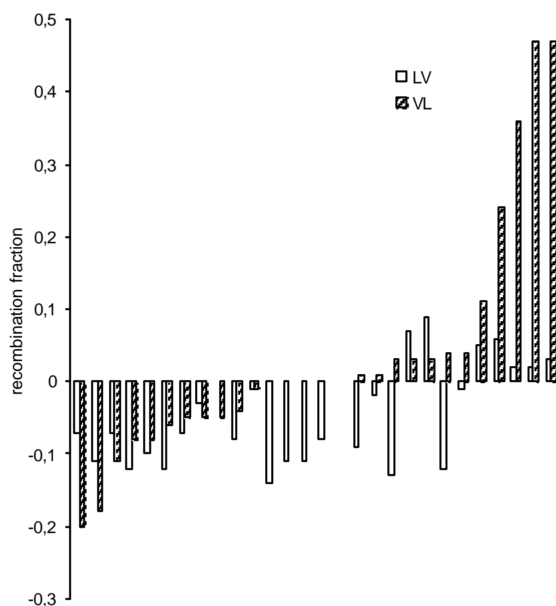

Figure 8: Deviations of recombination fractions of the reciprocal crosses LV and VL from the recombination frequency between eight microsatellite markers in the intraspecific *N. vitripennis* cross.

Table 4

Map statistics of the LV, VL and intraspecific map

	n	mapped loci	map length [cM]	mean interval [cM]	grouping [LOD score]	mapping [LOD score / rec.]
LV	94	56	193	3.45	7.0	5.0 / 0.35
VL	82	54	281	5.20	6.0	4.0 / 0.35
intraspecific	138	46	315	6.85	4.0	4.0 / 0.35

Table 5

Mapping efficiency of marker systems in the LV1, VL and intraspecific mapping population

	AFLP	microsatellite	RAPD	total
LV				
total	111	29	7	146
mapped	50	21	6	56
unmapped	61	8	1	90
mapping efficiency [%]	45	72	-	38
VL				
total	226	10	-	236
mapped	49	5	-	54
unmapped	177	5	-	182
mapping efficiency [%]	22	50	-	23
intraspedific				
total	233	12	-	245
mapped	46	11	-	57
unmapped	187	1	-	188
mapping efficiency [%]	20	92	-	23

In order to reduce the sampling error of locus order and map distance, shared markers of the reciprocal crosses LV and VL were pooled, since the homogeneity of multipoint recombination fractions and locus order were homogeneous between the reciprocal crosses (statistics Table 6). The shared marker map (LV_VL map) (Figure 939) was based on 176 individuals and 37 (27%) mapped loci out of 136 shared loci.

Table 6

Statistics of locus ordering map distance of the LV_VL map. Likelihood ratio test for homogeneity of locus ordering and multipoint map distance.

Chromosome	LLR	p	df	PCO [%]
1	0.00	1.00	8	87.5
2	0.00	1.00	7	59.0
3	0.00	1.00	5	45.5
4	0.00	1.00	5	30.3
5	0.00	1.00	12	14.8

LLR: log-likelihood ratio, df: degree of freedom, p: probability of chi-square distribution, PCO%: percentage of correct locus ordering on the basis of 100 bootstrap replicates of pooled data.

The average percentage of correct locus ordering differed between the linkage groups ranging from 87.5 % on linkage group 1 to 14.8 % on linkage group 5 (Table 6). Despite the differences in the average PCO value between linkage groups, this map was used as reference of locus ordering and map length for the LV1 and VL population. The order of shared markers of the LV and VL map correspond in most cases to the LV_VL map (Figure 9). In some cases, differences of locus order occured in distal chromosomal regions. The best resolution was achieved on linkage group 1 for the LV_VL map coinciding with locus ordering of the LV and VL map.

The LV and VL linkage groups were smaller than the corresponding LV_VL linkage group except linkage group III. Although sample size and the number of mapped markers were similar between the reciprocal crosses, the VL map showed always a greater map expansion than the corresponding LV linkage group.

Due to the set of chromosomal anchored microsatellite markers that had been mapped with markers developped from microdissected chromosomes (Ruetten et al. 2004) (Table 7) and shared markers between the LV1 and VL map, linkage groups could be assigned to specific chromosomes (see Figure 5, p. 34).

Table 7
Chromosomal anchored microsatellites mapped in a LV cross with markers developed from microdissected chromosomes

chrom 1	chrom 2	chrom 3	chrom 4	chrom 5
Nv-22	Nv-20	-	Nv-21	Nv-25
	Nv-23		Nv-24	Nv-27
	Nv-26		Nv-37	

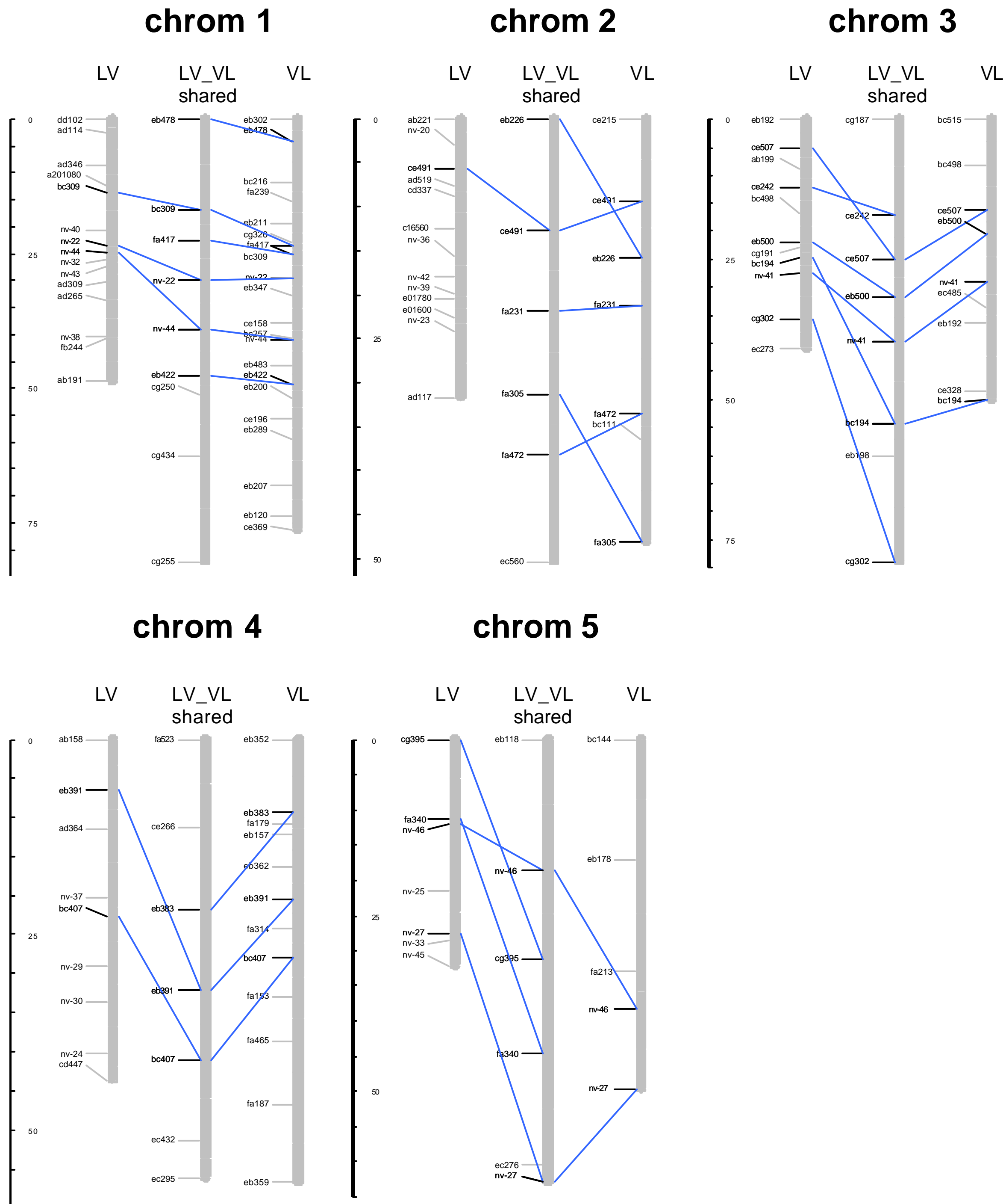

Figure 9: Map comparison between the LV and VL map with the map based on shared markers between the reciprocal crosses (LV_VL map). Shared markers are indicated with a red signature and are connected with dotted lines. Vertical ruler indicate map size in centi morgan [cM].

Genome interactions based on marker statistics

In the absence of hybrid incompatibilities, nuclear genes are supposed to be randomly shuffled in the hybrid genome resulting in a 1:1 ratio of the parental alleles. However, segregation distortion in hybrid genomes may either result from nuclear-nuclear (nuc-nuc), nuclear-cytoplasmic (nuc-cyt) or nuclear-nuclear-cytoplasmic (nuc-nuc-cyt) incompatibilities (IC) between heterospecific alleles.

The recovery of *N. vitripennis* (ASYMC) alleles in F_2 hybrid males were normally distributed in all three investigated crosses (*Shapiro-Wilk* **intraspecific**: $W = 0.99$ $p = 0.93$; **LV**: $W = 0.97$, $p = 0.26$; **VL**: $W = 0.98$, $p = 0.7$) (Figure 10). A deviation from the average recovery rate of a proportion of 0.5 *N. vitripennis* (ASYMC) alleles was used as an indication of nucleo-cytoplasmic incompatibilities on a global scale. In both interspecific crosses (LV, VL) the mean recovery rate was significantly biased towards the maternal genome, whereas the recovery rate in the intraspecific cross did not deviate from an expected 0.5 proportion of *N. vitripennis* (ASYMC) genome (*t-test* against 0.5 *N. vitripennis* (ASYMC) genome: **intraspecific**: $t = -1.07$, $p = 0.29$, $FG = 53$; **LV**: $t = 8.98$, $p = 0.0000001$, $FG = 55$; **VL**: $t =-6.99$, $p = 0.0000001$).

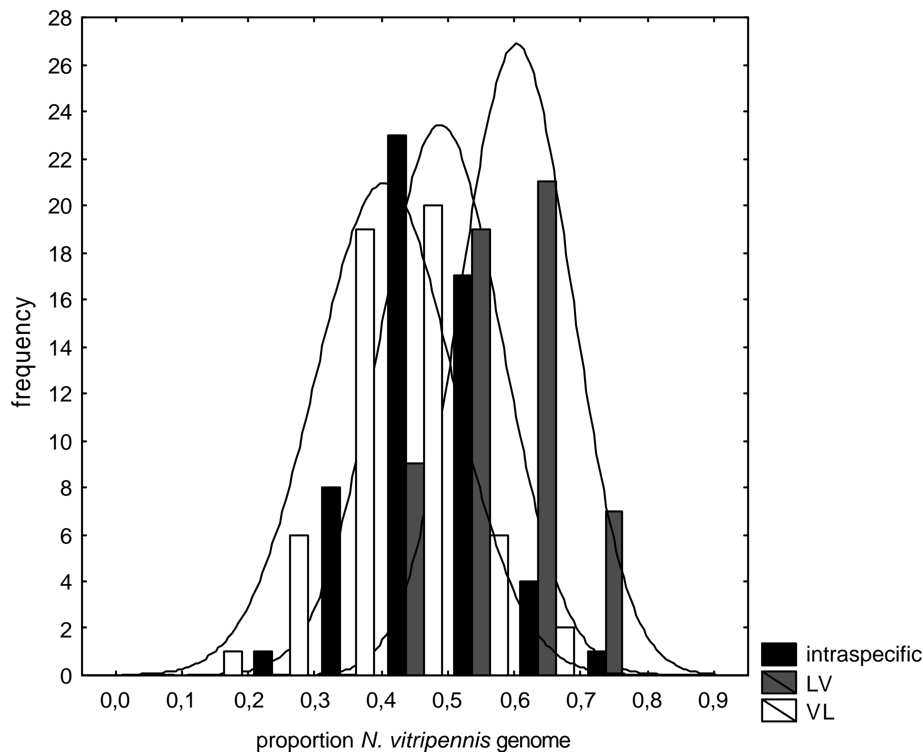

Figure 10 Recovery rate of *N. vitripennis* genome in F_2-hybrid males of the intraspecific, LV and VL cross.

In the analysis of individual segregation ratios of shared markers between the reciprocal crosses, alleles of a marker fraction deviated from a 1:1 ratio towards the respective maternal alleles (Figure 11 red dots) and hence appear to interact negatively with the heterospecific cytoplasm, i.e. the frequency of the *N. vitripennis* alleles was significantly increased in the LV cross with the *N. vitripennis* cytotype and significantly decreased in the VL cross with the N. *longicornis* cytotype. However, some markers showed a bias towards the maternal allele in one cross but did not deviate from an equal segregation ratio in the reciprocal cross (green dot). A set of markers showed a balanced ratio in the LV cross, but showed a deficiency of *N. vitripennis* alleles (**A** Figure 11). Another fraction showed a bias towards the N. vitripennis alleles in the LV cross but showed no deviation from a 1:1 ratio in the reciprocal VL cross (**B** Figure 11) indicating an influence of of the cytotype of the segregation ratio only in one cross. Unexpectedly, a number of markers even showed a biased segregation ratio in both crosses towards the *N. vitripennis* allele (yellow dots).

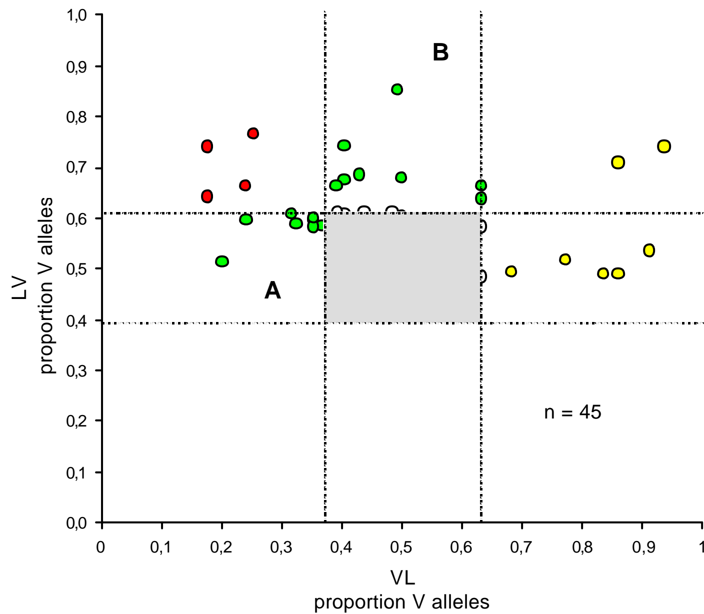

Figure 11: Segregation ratio of shared and mapped markers of the LV and VL cross. Dotted lines demarcate χ^2-limits for a 1:1 ratio calculated on the mean segregation ration of all shared and mapped markers.

To visualize differences of segregation patterns and their localization in different genetic backgrounds, the segregation ratios for shared markers of both reciprocal crosses were plotted on the LV_VL map (Figure 12). The comparison revealed different degrees of discrepancies in the reciprocal crosses. In seven out of 42 markers the segregation ratio in the LV cross was biased towards the *N. vitripennis* genome and reciprocally biased towards the *N. longicornis* genome in the VL cross indicating nucleo-cytoplasmic interactions occurring in the hybrid genome indicated with (2) at the marker position (see Figure 12). Unidirectional nuc-cyt IC was deteted over all linkage groups in 49 % of all markers, i.e. the segregation ratio was biased in one cross but did not deviate from a 1:1 ratio in the reciprocal cross (1). One third of all mapped markers showed the same segregation ratios in both reciprocal crosses (0).

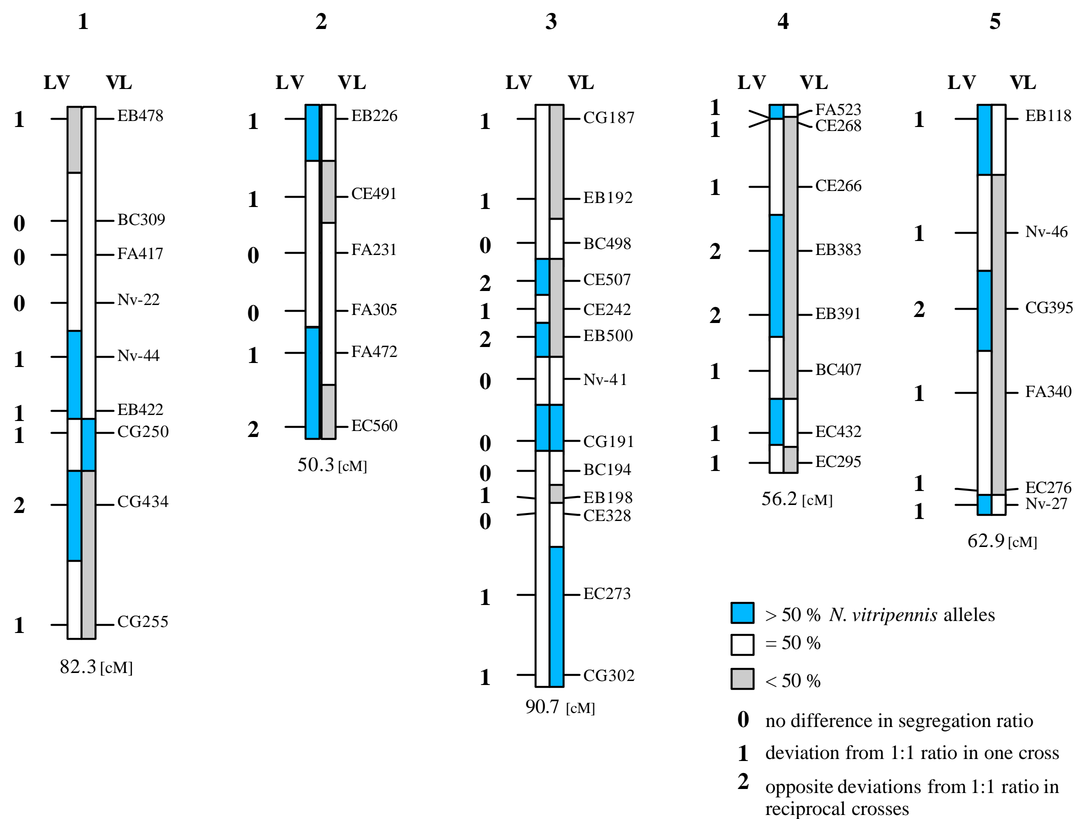

Figure 12: Comparison of segregation ratio of *N. vitripennis* alleles in an LV and a VL cross. Map based on shared markers of both mapping populations. Significant deviations from a 1:1 ratio were calculated with a χ^2-statistic.

In a further analysis, hybrid incompatibilities had been detected through linkage disequilibrium (LD) between alleles of two loci in the LV and VL cross. Nuclear-nuclear hybrid incompatibilities can be detected by a significant excess of conspecific haplotypes and a deficiency of heterospecific haplotypes between two loci seen in Table 8.

Table 8

Deficiency of heterospecific in two-locus haplotypes in one and two haplotype classes (shaded areas).

E011000	E01600 V	E01600 L	
V	25	23	48
L	0	14	14
	25	37	62

Nv-39	Nv-46 V	Nv-46 L	
V	40	11	51
L	13	26	39
	53	37	90

V: *N. vitripennis* allele, L: *N. longicornis* allele; Nv-39, Nv-46: loci for which significant linkage disequilibrium has been detected.

Seventeen significant LD had been detected in the LV cross on the basis of a Dunn-Sidak corrected type one error (a*= 0.0002, 233 significant LD on the basis of 0.05). In the VL cross the type one error was adjusted to a * = 0.00003 to account for 626 significant LD. In most cases, significant interchromosomal LD was detected for multiple adjacent markers so that hybrid incompatibilities could often not be pinpointed to single marker combinations. Loci that are involved in hybrid incompatibilities have been detected on all five chromosomes in both reciprocal crosses (Figure 13, Figure 14). Nuclear-nuclear three-way interactions have been identified through shared markers of two marker-pairs with significant linkage disequilibrium.

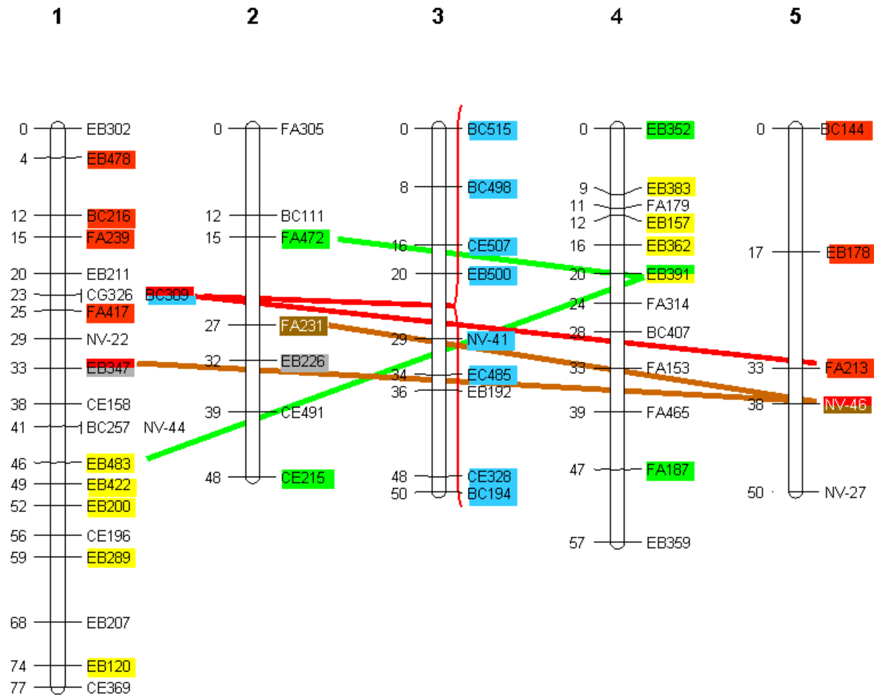

Figure 13: VL map with interchromosomal hybrid incompatibilities detected with significant linkage disequilibrium between alleles of two loci. Colours indicate non-random allele association of loci between specific chromosomes (e.g. yellow: interaction between chromosome 1 and four). Identified three-way interactions are indicated with connecting lines.

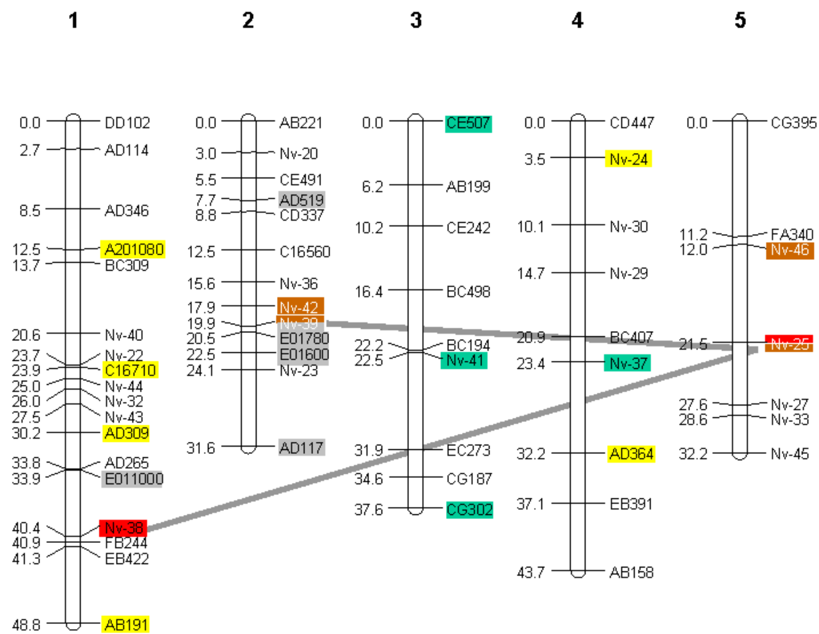

Figure 14: LV map with interchromosomal hybrid incompatibilities detected with significant linkage disequilibrium between alleles of two loci. Colours indicate non-random allele association of loci between specific chromosomes (e.g. yellow: interaction between chromosome 1 and four). Identified three-way interactions are indicated with connecting lines.

Table 9

Contingency tables of haplotype frequencies of two loci in the LV and VL cross. Example of maternal haplotype frequency resulting from nuclear-cytoplasmic incompatibilities

VL	EC432			**LV**	EC432		
EB500	V	L		EB500	V	L	
V	16	13	29	V	42	14	56
L	15	35	50	L	17	16	33
	31	48			59	30	

EB 500 and EC432: AFLP markers, V: *N.vitripennis* allele, L: *N. longicornis* allele

In another approach, LD was calculated only for shared markers that had been mapped on the LV_VL map in both reciprocal crosses in order to compare haplotype fequencies in different cytoplasmic backgrounds, i.e. the *N. vitripennis* or *N. longicornis* cytotype (e.g. Table 9). The type one error was corrected for 673 significant LD (a<0.05) in the VL cross (a*= 0.000008) and for 215 significant LD in the LV cross (a*= 0.0002).

Only marker-pairs that showed significant LD in both reciprocal crosses were used for further analysis. From 44 marker combinations that showed significant LD in both reciprocal crosses, six hybrid incompatibilities could be extracted, that were present in both reciprocal crosses (Figure 15). In most cases, in which an excess of the maternal haplotype had been detected in the VL cross, nuclear-nuclear incompatibilities as well as nuclear-cytoplasmic incompatibilities were involved, whereas in the LV cross nuclear-nuclear incompatibilites were almost absent except in the case of the marker combination CE491 - CG395 (Figure 15).

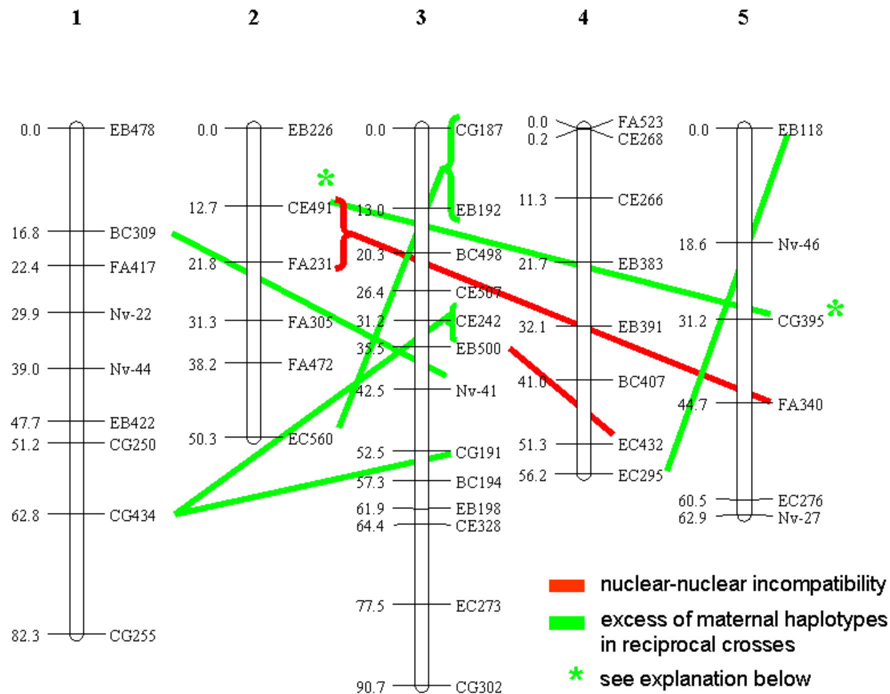

Figure 15: Shared hybrid incompatibilities in the LV and VL mapping population on the LV_VL map. Excess of maternal haplotypes was mostly effected by nuc-cyt IC in the LV cross, but resulted from a combined effect of nuc-nuc IC and nuc-cyt IC in one case (*) whereas in the VL cross the combined effect of nuc-nuc IC and nuc-cyt IC waa found to be common.

Phenotypic analysis

Courtship behaviour

The phenotypic analysis of courtship behaviour was based on 480 phenotyped individuals. Under our experimental paradigm a large proportion (about 70%) of LV hybrid males did not succeed in courting. They were either not able to get in a proper courtship position or did not approach the female in the right way.

Phenotypic distributions of all courtship components were skewed. Chronological traits as latency, fix-1st nod, cycle time and the total number of series were lognormally distributed (*Kolmogorov-Smirnov*, cycle 1st-4th, latency, fix-1st nod, total number of series: D = 0.75, 0.85, 0.91, 0.96, 0.90, 0.61, 0.84; *Lilliefors-p* < *n.s.*, n = 460, 459, 296, 283, 407, 391, 282). Due to the skeweness of the phenotypic distribution only median and the interquartil range are shown (Table 10). The frequency distribution for number of head nods were Poisson-

distributed (*Kolmogorov-Smirnov*, **head nod 1st-4th**: $D = 0.26, 0.29, 0.27, 0.27$; *Lilliefors-p* < *n.s*, $n = 477, 479, 474, 451$).

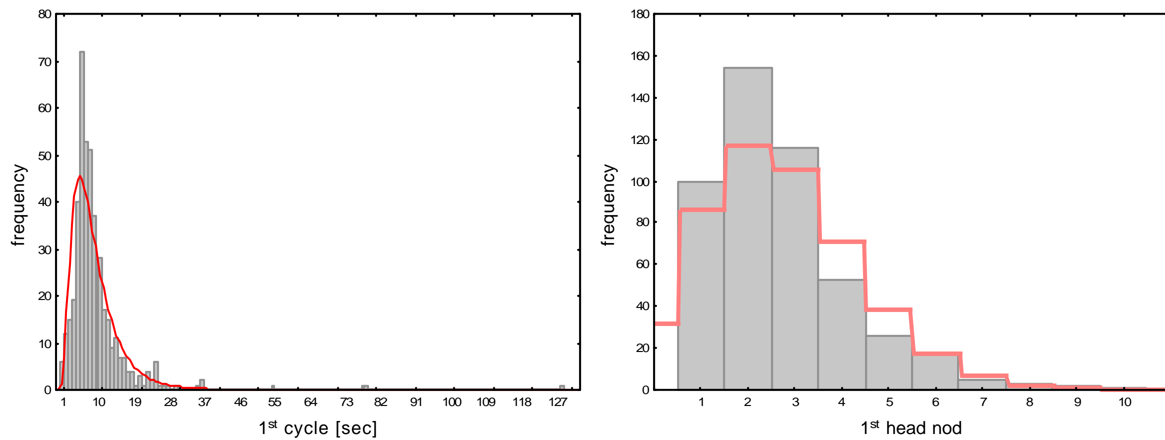

Figure 16: Phenotypic distribution of the cycle time (n=460) and the number of head nods (n = 477) in the first series in the LV hybrids.

Table 10
Descriptive statistics of male courtship components of LV hybrids and parental strains

	N	Median	Interquartile range	min	max
LV[V] hybrids					
latency	375	120.0	132.5	0.0	300.0
fix 1st nod	391	4.0	5.2	0.1	70.0
1st cycle	461	8.0	5.1	1.4	129.0
2nd cycle	459	9.0	5.0	1.6	67.7
3rd cycle	297	9.0	4.9	1.2	44.6
4th cycle	284	9.1	4.8	1.5	25.2
1st head nod	478	2.0	1.0	1.0	10.0
2nd head nod	479	3.0	2.0	1.0	10.0
3rd head nod	475	3.0	2.0	1.0	12.0
4th head nod	452	3.0	2.0	1.0	11.0
mean head nod	477	2.8	1.8	1.0	10.0
h2-h1	479	0.0	1.0	-4.0	6.0
total series	283	11.0	8.0	3.0	73.0
Parental lines *N. vitripennis / N. longicornis*					
1st cycle	68/79	7.0/12.0	2.0/3.0	4.0/9.0	11.0/22.0
1st head nod	93/79	5.0/1.0	1.5/1.0	3.0/1.0	10.0/2.0
total series	82/45	7.5/9.0	2.0/4.0	5.0/5.0	15.0/20.0

Cycle time, number of head nods in the first series and latency of the hybrid courtship display differed significantly from both parental phenotypes (*Kruskal-Wallis* 1st cycle, 1st head nod, h2-h1, total number of series, $H = 94, 198, 83, 33$, $p < 0.001, 0.001, 0.0001$,

0.0001, $n = 608, 650, 620, 620$; ?² goodness of fit, latency ?² = 73-102, $p < 0.01$, 2 *df*, n = 425-429). Except for cycle time a general bias towards the grand paternal *N. longicornis* behaviour was detected. The phenotypic distributions for 1st cycle, 1st head nod, h2-h1, and the total number of series showed no significant differences in their dispersion between *N. vitripennis* and *N. longicornis* (*Mood rank dispersion* 1st cycle, 1st head nod, h2-h1, total number of series, u = 1.13, 1.4, 0.39, 1.39; p = *n.s.*, 0.0001, *n.s.*, *n.s.*, n_1 =68, n_2 = 79; n_1 = 79, n_2 = 93; n_1 = 45, n_2= 82; n_1 = 64, n_2 = 79). For that reason the parental variances were combined because the variance occuring in the parental strains can be used to estimate the QTL effect in environmental variance (see below).

Head nods and cycle time in the four consecutive series were significantly concordant (*Kendalls'* coefficient of concordance two-tailed, corrected for ties: $W = 0.085$, $z = 1.86$, $p < 0.01$, $n = 446$, for the number of head nods; W= 0.227, Fisher $z = 2.18$, $p < 0.01$, $n = 267$ for cycle time). In nearly all cases chronological courtship components were positively correlated. Latency was significantly correlated with fix-1st nod and mean cycle[8] time at the 0.001 level to (Spearman's ? = 0.24, $n = 307$; ? = 0.22, $n = 374$) and so was fix-1st nod with mean cycle time (? = 0.52, $n = 391$). The statistical association of head nods with other continuous courtship components were tested by splitting up the hybrid population into two classes according to whether they showed a *N. vitripennis* or *N. longicornis*-like number of head nods (see Materials & Methods). Only cycle time in all four cycles differed significantly between the two groups (*U*-Test 1st cycle $Z = 5.8$, $p < 0.00001$, $n = 344$). We found a coupling of higher number of head nods with long cycle time in the hybrid males in contradiction to the parental phenotypes, i.e. higher number of head nods with shorter cycle time in *N. vitripennis*. Minus nods and forefeet rubbing which are unique to *N. longicornis* were significantly correlated (?² = 5.58, $p < 0.05$, 2 *df*, $n = 390$), although only about 15 % of the hybrid males

[8] The mean cycle time was calculated from the first four cycles in order to simplify the description of phenotypic correlations.

showed both behaviours, whereas about 40 % of the LV[V] hybrid males showed neither of these traits.

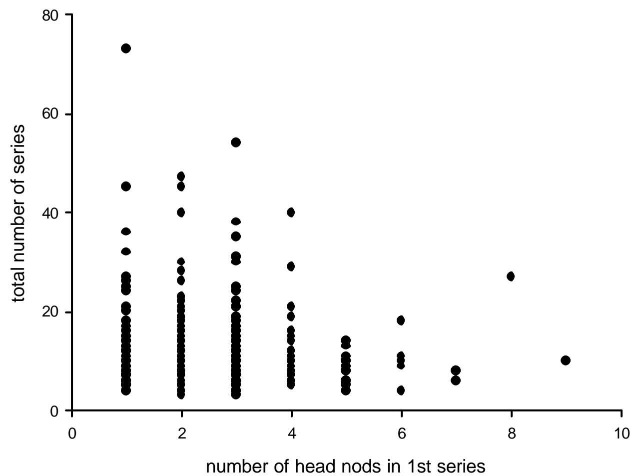

Figure 17: Relation between total number of series and the number of head nods in the 1st series in hybrid courtship displays (n=283).

Head nods in the 1st series were not significantly correlated with the total series; hybrid males that show a low number of head nods may show a short or a long display, whereas individuals with a high number of head nods in the 1st series are more likely to show a shorter display on average (Figure 17).

The phenomenon that hybrid phenotypes stand outside the range of parental phenotypes has been referred to as transgression of the phenotype and it is regularly observed in hybrids (Rieseberg et al 1999, Rieseberg et al. 2000). Individuals with transgressive behaviour were found for cycle time (5%) and the total number of series (13 %) (Figure 18).

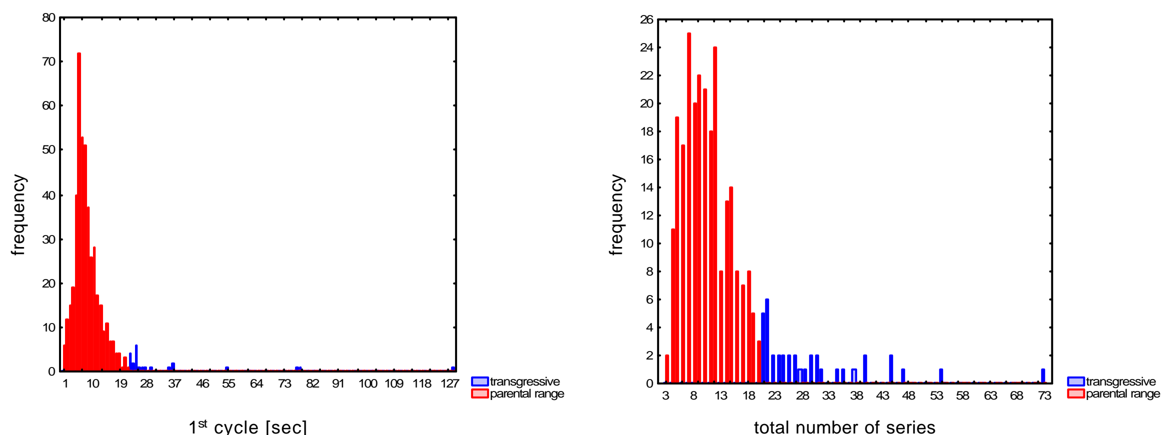

Figure 18: Phenotypic distribution and transgressive phenotypes for cycle 1st (n = 461) and the total number of series (n = 283).

Behavioural sterility

In the functional cycle of courtship *Nasonia* males actively search for mates. After mounting, they immobilize the female and take place in the specific courtship position on the females head and thorax before they start courting. A proportion of a total of 1600 observed hybrid males of the LV cross showed behavioural dysfunctions (Figure 19). Seventy percent of hybrid males did not achieve to court a female during five minutes. Sixty percent of these males did not show searching behaviour at all. The remaining proportion of non-courters showed different behavioural dysfunctions. A proportion of 90 % of the non-courting males seemed to shy upon the female during mate searching behaviour and therefore did not achieve to court within five minutes. A proportion of 10 % of non-courting males were not able to take place in the right courtship position. They either turned around on the female and did not start courting or they courted in the opposite direction in a distal position. Five percent of hybrid males that performed a courtship display were not able to immobilize the female. In those instances, males additionally showed intense wing vibrations. In most cases where behavioural dysfunctions have been observed, the behavioural sequence was disturbed in several respect, e.g. a hybrid male that was not able to orientate in the right courtship position took several attempts to mount the female.

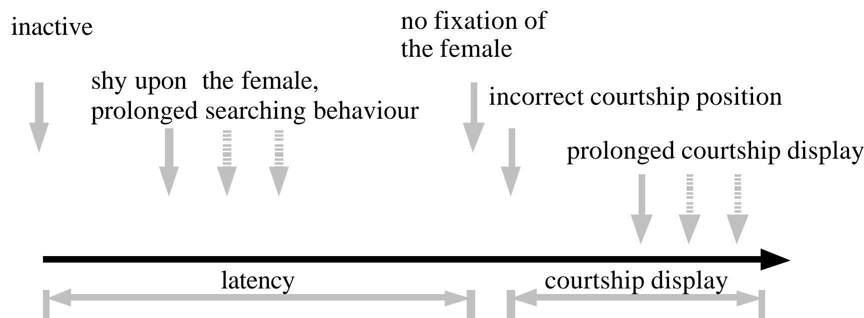

Figure 19: Behavioural dysfunctions of LV hybrid males in the behavioural sequence of mate searching behaviour and courtship display.

Wing size

Morphological characters as wing size are at least partially related to body size. Therefore the interocular distance was used as a body size parameter because it has been shown that the interocular distance is correlated with relative body size in *Nasonia* (Skinner 1983, see also Gadau et al. 2002, Weston et al. 1999). Nineteen percent of wing length and nine percent of wing width can be explained by body size (linear regression **wing length**: r = 0.43, p = 0.00004, r^2 = 0.19, n = 87; **wing width**: r = 0.31, p = 0.0054, r^2 = 0.09, n = 83) (Figure 20). The seta density was not significantly affected by body size, so that this measure was not corrected for body size (linear regression **seta density**: r = 0.02, p = 0.87, r^2 = 0.0003, n = 86).

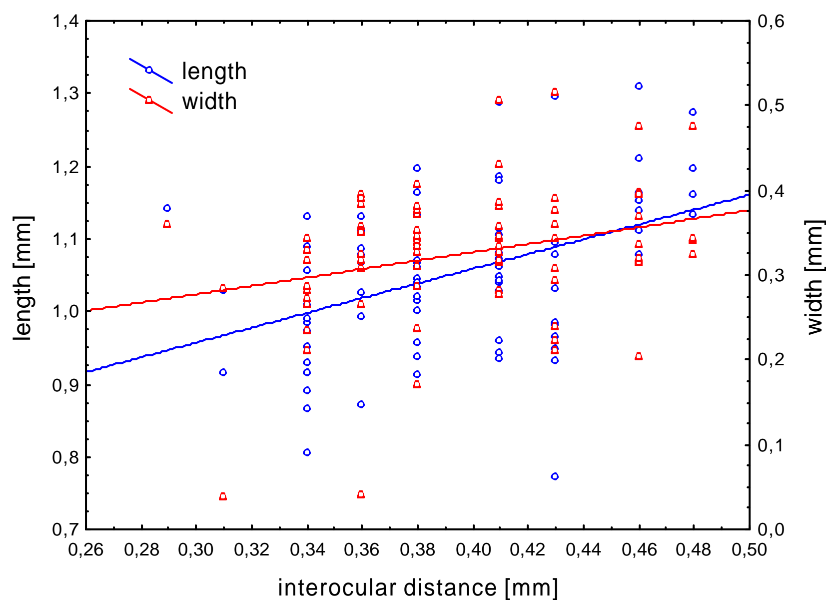

Figure 20: Linear regression of wing length and wing width against interocular distance as a measure of relative body size

All traits were normally distributed except wing width that failed the non-significance threshold (*Kolmogorov-Smirnov*, **length (normalised), width x length (normalised), seta density, width (normalised)**: D = 0.07, 0.07, 0.06; 0.11, *Lilliefors-p* < *1, p = 0.01*, n = 83, 83, 86, 87). Wing length and wing width were highly significantly correlated, whereas the correlation among other wing size measures were distinctly lower or even failed significance (Table 11). Only the courtship component cycle time showed significant correlations to wing width related traits except for wing length.

The median of wing measures of the LV hybrids were intermediate between the parental strains (Table 12) but were significantly biased towards the *N. vitripennis* phenotype

(*Kruskal-Wallis* **wing x length (norm), length (norm), width (norm),** $H = 34, 23, 32$, $p < 0.0000$, $n = 117, 113, 117$). The wing measures of both parental strains showed no significant differences in their dispersion, so that a combined variance for *N. vitripennis* and *N. longicornis* could be calculated as the standing environmental variance (*Mood rank dispersion* **wing length (norm), wing width (norm), wing x length (norm):** $u = 1.2, 1.1, 1.1$; $p = n.s.$, $n_1 = 15$, $n_2 = 15$).

A transgression of phenotypes was observed towards reduced wing sizes and hence towards the maternal *N. vitripennis* phenotype in contrast to courtship components that show a transgression towards the *N. longicornis* phenotype. The proportion of transgressive individuals ranged from 22% for wing width to 36 % for wing length.

Table 11

Phenotypic correlation (Spearmans' rank correlation) among wing size measures and courtship components. (Only significant correlations are reported)

wing measures *vs.* wing measures	n	r^s	p-value
width (norm.) *vs.* length (norm.)	83	0.58	0.000
seta density *vs.* width (norm.)	85	-0.26	0.017
seta density *vs.* width x length (norm.)	81	-0.23	0.039
wing measures *vs.* courtship components			
seta density *vs.* 1st cycle	77	-0.27	0.019
seta density *vs.* 2 nd cycle	70	-0.34	0.004
width x length (norm) *vs.* 1st cycle	74	0.27	0.021
width_(norm) *vs.* 1st cycle	78	0.32	0.0045
width_(norm) *vs.* 2 nd cycle	71	0.28	0.0166

Table 12
Descriptive statistics of wing measures of LV hybrids and parental strains.

	n	median	min	max	interquartil range
LV[V] hybrids					
length (norm)	83	2.7	1.8	3.9	0.4
width (norm)	87	0.8	0.1	1.2	0.2
width x length (norm)	83	2.2	0.3	4.9	0.9
seta	86	98.5	47	145	24
Parental lines *N. vitripennis / N. longicornis*					
length_(norm)	15/15	2.7/3.0	2.6/3.0	3.3/4.0	0.3/0.1
width_(norm)	15/15	0.8/1.1	0.7/1.0	1.2/1.8	0.1/0.1
length x width (norm)	15/15	2.1/3.4	1.8/3.1	3.9/7.0	0.5/0.3

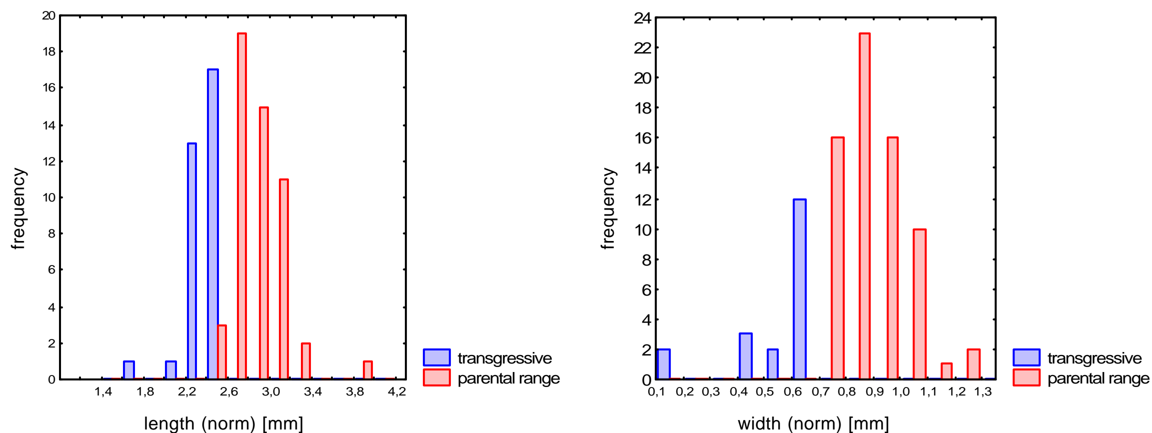

Figure 21: Phenotypic distribution and transgressive phenotypes for wing length (n = 76) and wing width (n = 80).

QTL analysis

A QTL analysis based on 94 individuals from an LV cross (1st set) revealed 14 QTL for eight recorded traits (Table 13). QTL for traits that had been scored in consecutive cycles, e.g. cycle time or head nods were combined when they mapped in the same region (Figure 22). The total proportion of the phenotypic variance that could be explained by the detected QTL for a trait varied between 15 to 90 % (Table 13, column 2). The best resolution was achieved for cycle time. The QTL model could explain up to 90 % of the phenotypic variance

for cycle time in the fourth cycle. For another chronological trait fix-1st nod only one QTL could be mapped on chromosome 5, which coincided with a cycle time QTL. The total QTL model based on that QTL could only explain fifteen percent of the phenotypic variance occurring in the hybrid mapping population. Although latency is supposed to be highly environmentally influenced owing to the female's activity two latency QTL have been mapped on chromosome 1 and 2. Head nod QTL could be detected on chromosome 2 and 5 that account for maximal 42 % of the phenotypic variance in the fourth series. One QTL for the binary trait 'minus nods' mapped in vicinity to a head nod QTL on chromosome 2 and the other binary trait 'forefeet rubbing' mapped to chromosome 3 and 5. The composed trait h2-h1 that has been used to map the *N. vitripennis* specific decline of head nods in the second series relative to the first series could not be mapped at all.

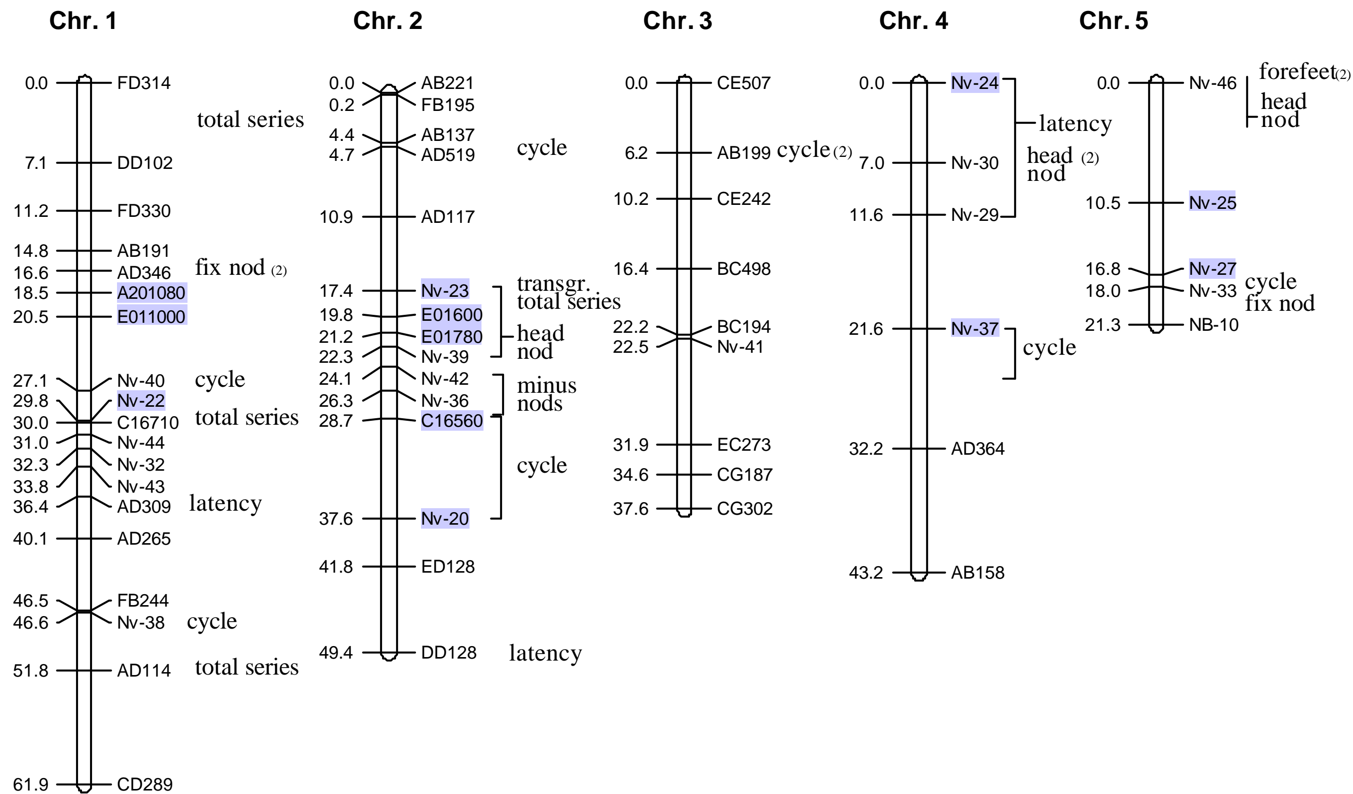

Figure 22: QTL analysis for male courtship components based on a mapping population of 94 LV hybrid males. Vertical bars embrace regions where QTL location for cycle time or head nods in consecutive cycles mapped to slightly different map locations. QTL that were nexly detected in an idependent mapping population (2nd set) were marked with (2). Grey underlaid markers have been assigned to specific chromosomes (see Figure 7, p. 35)

Table 13
Significant courtship QTL based on a LV mapping population of 94 individuals and 56 mapped markers (1st set).

trait	total expl. var. [%]	LOD a <0.05[a]	chr.[b]	LOD / p*[3]	associated marker / interval	mean N. vit.	mean N. long.	expl. var. [%]	MQM - cofactor
cycle 1st	42.8	1.8	1	3.3	Nv-40	112.8	172.0	16.9	Nv-40, Nv-33
			2	2.9	Nv-20	8.0	21.0	13.7	
			5	2.5	Nv-33	8.2	20.4	12.2	
cycle 2nd	82.2	2.1	1	6.8	Nv-38	8.9	22.5	28.7	Nv-38, Nv-33
			2	4.0	C16560	8.3	17.8	17.9	
			3	2.8	Nv-29, Nv-37	9.1	17.8	14.4	
			5	4.7	Nv-33	8.2	18.6	21.2	
cycle 3rd	88.6	2.2	1	5.9	Nv-38	8.9	18.8	29.3	Nv-38, Nv-33
			2	2.7	Nv-20	9.1	15.5	14.8	
			3	2.4	NV-37, AD364	9.0	16.5	19.4	
			5	4.8	Nv-33	8.2	16.2	24.7	
cycle 4th	86.7	2.3	1	11.0	Nv-40	9.0	15.8	39.0*[1]	Nv-40, Nv-33
				5.8	AD265, FB244	10.7	15.0	5.1*[1]	
			3	2.3	Nv-37, AD364	9.2	14.6	20.3	
			5	4.1	Nv-33	8.8	14.0	22.3	
hnd 1st	21.0	2.3	2	4.5	E01780	2.0	4.1	21.0	Nv-39
hnd 2nd	31.3	2.3	2	3.6	Nv-39	2.4	4.0	16.2	Nv-39
			5	3.3	Nv-46	2.4	4.0	15.1	
hnd 3rd	31.4	2.2	2	3.5	Nv-39	2.5	4.0	16.1	Nv-39
			5	3.4	Nv-46	2.5	4.1	15.3	
hnd 4th	42.6	2.3	2	2.6	E01600	2.6	4.0	12.8	Nv-39
			5	5.7	Nv-46, Nv-25	2.4	4.6	29.8	
latency	28.8	2.3	1	2.4	AD309	107.8	188.7	12.0	AD309
			2	3.5	DD128	107.3	208.8	16.8	
f1x 1stnod	15.1	2.0	5	2.5	Nv-33	4.1	12.7	15.1	Nv-45
total series	69.9	2.3	1	5.8	AD114	6.5	20.0	26.0*[1]	AD114
				5.1	Nv-32	20.3	6.0	23.9*[1]	
				3.2	FD314,DD102	5.9	18.7	20.0*[1]	
total series (transgressive)*[2,3]			2	0.001*[3]	Nv-23				
minus nods*[3]			2	0.014*[3]	E01700				
forefeet rubbing*[3]			5	0.025*[3]	Nv-46				

[a] genome wide threshold
[b] chromosome number.
*[1] Explained variance are shown for MQM mapping when more than on QTL per chromosome present
*[2] The phenotype was classified as transgressive, when the number of series exceeded twenty series.
*[3] Binary trait detected through linkage disequilibrium (LD) with Fisher's exact test. Type I error adjusted by the number of significant association of binary trait and marke r alleles.

Multiple peaks for cycle time appeared on chromosome 1 in interval mapping. After including the marker associated with the QTL of highest effect, two cycle time QTL on chromosome 1 could be detected (Figure 23A). MQM uncovered three QTL for the total

number of series on chromosome 1 (Figure 23B). The middle QTL showed a complementary gene action, i.e. an allele reducing a trait derived from the strain with the highest phenotypic value and vice versa. This QTL masked the presence of the two additional QTL in interval mapping. The LOD scores for the three independent QTL were significantly higher than the LOD score for the single QTL detected by interval mapping (3.2 - 5.8 versus 2.5, Table 13).

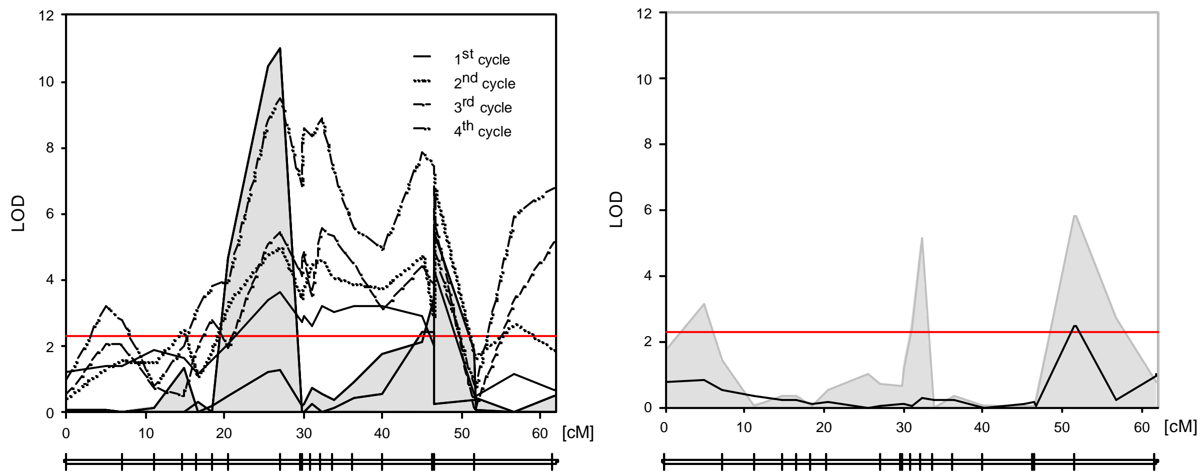

Figure 23A) LOD plot for cycle time A) and the total number of series B). Lines represent the LOD plots of interval mapping. The shaded area in A) represents the combined MQM LOD plots for all four cycles. Marker positions are indicated on the linkage group below the plots. The horizontal line indicates the genome wide LOD score threshold with a =5 % inferred by permutation test.

The binary trait ‘transgressive total series’ was found to be associated with marker Nv-23 chromosome 2 (Fisher’s exact $p = 0.0001$). The allelic effects acted in the opposite direction than expected. In the hybrids the *N. longicornis* allele was associated with a lower number of total series, although the parental *N. longicornis* line shows higher number of series on average than the *N. vitripennis* line. A reversed allelic effects has also been found for head nod QTL on chromosome 2 and 5. These QTL could explain up to 30 % of the phenotypic variance (Table 13). The reversion of allelic effect was already mirrored in the phenotypic analysis, where low numbers of head nods were associated with short cycle times in contradiction to the behaviour of the parental strains.

In a second independent experiment based on 94 individuals (2nd set) three QTL locations for cycle time and fix-1st nod could be confirmed (Table 14). QTL for latency and

head nods on chromosome 2 and 5 could not be recovered, but a new QTL for fix-1st nod and for forefeet rubbing have been identified on chromosome 1. However, differences in the amount of detected markers may be partially caused by fewer genotyped markers in the second set!

All wing size QTL were associated with unmapped markers. Therefore, only four wing size QTL have been assigned to specific chromosomes based on linkage analysis with previously mapped markers from the map in Figure 22 (Table 14).

Summing up, both QTL analyses revealed a complex genetic architecture of different courtship components and wing size differences, but the resolution differed between the traits. Only a set of several QTL for chronological traits like cycle time and fix-1st nod that had been detected in the first analysis could be recovered in the second independent mapping experiment.

Table 14

Significant courtship QTL based on a LV mapping population of 94 individuals and 46 mapped markers (2nd set).

trait	total expl. var. [%]	LOD a <0.05[a]	chr.[b]	LOD	associated marker / interval	mean N. vit.	mean N. long.	expl. var. [%]	MQM - cofactor
fix 1st nod	38.2	1.8	1	3.5	AD346	5.2	15.5	21.3	
			5	2.7	Nv-27*[1]	4.8	12.8	16.9	
cycle 1st	48.7	1.7	1	3.7	CD346*[2]	10.3	17.6	10.2*	Nv-40
			1	2.9	Nv-40*[1]	9.0	16.2	14.0*	
cycle 2nd	52.4	2.9	1	3.6	Nv-40*[1]	10.1	15.7	18.2	Nv-40
			1	3.4	CD346*[2]	11.2	16.5	9.7	
			5	4.1	Nv-27*[1]	9.6	15.9	24.1	
head nod 1st	35.0	2.2	4	2.3	Nv-30	3.3	2.1	11.8	
wing length x width	22.0	2.3	4	3.5	Nv-21*[2]	2.0	2.7	22.0	
wing width	28.7	2.3	4	5.2	Nv-21*[2]	0.8	1.0	28.7	
seta density	16.0	2.3	1	2.6	CD346*[2]	101.3	84.3	16.0	

[a] genome wide threshold
[b] chromosome number
*[1] QTL that has been already detected in the first QTL analysis (1st set)
*[2] Chromosomal assignment of marker by highly significant two-point linkage (LOD 7.0) to a mapped marker

QTL effect and sample size

To assess effect of the sample size on the estimated QTL effect, an experiment of five QTL analysis with increasing sample size ranging from 94 to 270 individuals was performed for the cycle time in the second cycle. The linkage map was based on 206 AFLP marker and 326 LV hybrid males (Figure 26). A total of 54 markers mapped to six linkage groups, from which five could be assigned to specific chromosomes. The mapping efficiency of AFLP markers amount to 26 %. The estimated QTL effect was drastically affected by sample size. The largest effects yielded the QTL analysis with 94 individuals. For instance, the estimated phenotypic effect of a cycle time QTL on chromosome 1 (Figure 24, left axis) decreased from 23 to 10 % explained phenotypic variance with increasing sample size. At the sample size of 180 individuals, the phenotypic effect oscillated around 10 % explained variance. The percentage of phenotypic variance that could be explained by the entire QTL model showed a similar trend. In contrast, the number of unmapped markers that showed a significant QTL effect increased as well as the amount of their total phenotypic effect with sample size (Figure 25).

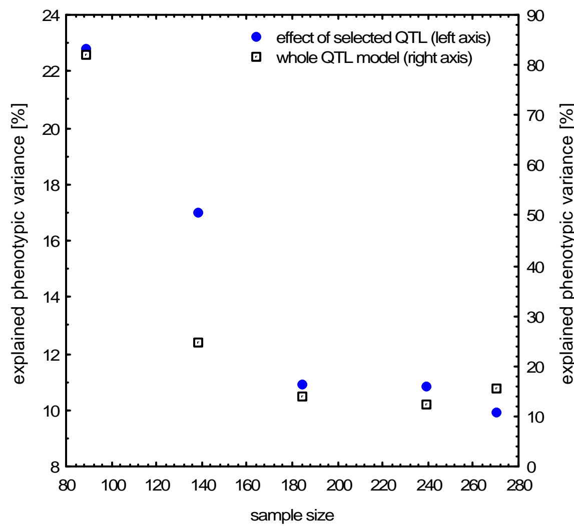

Figure 24: Dependence of the estimated QTL effect expressed in explained phenotypic variance on sample size for two QTL for cycle time in the 2nd cycle (left axis) and the explained variance of the set of significant QTL for cycle time in the 2nd cycle (right axis)

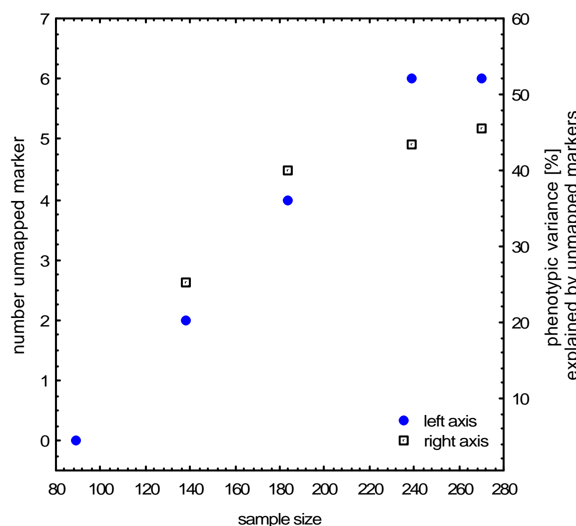

Figure 25: Dependence of the number of unmapped markers with significant QTL effect for cycle time in the 2nd cycle (left axis) and their estimated phenotypic effect (right axis).

The QTL analysis for courtship components based on 298 LV hybrids revealed 26 QTL for courtship components (Figure 26 ,Table 15). Six QTL were new and had not been detected in the initial QTL study (1st set). But eleven QTL, that had been previously mapped, could not be confirmed in the final QTL analysis with the extended sample size. This applied especially for mapped head nod QTL that showed a reversed allelic effect in the initial study. In the final QTL analysis all mapped head nod QTL showed an allelic effect according to the parental behaviour, although a set of unmapped markers still showed that reversed effect (appendix 10). Significant linkage disequilibrium for the binary trait 'minus nods' has been found nearly over the entire range of chromosome 2 and newly on chromosome 1 and 5. The other binary traits 'forefeet rubbing' and 'transgressive total number of series' could not be detected through significant linkage disequilibrium.

In the final QTL analysis QTL locations for different courtship components coincided more frequently than in previous QTL analysis based on smaller sample sizes. For instance, QTL for cycle time, fix 1st nod and latency mapped to the same locus on chromosome 1 and 5. However, the confidence intervals for QTL locations calculated according to the formula (*confidence interval [cM] = 530 / sample size x QTL effect [%]*) from Darvasi and Soller (1997) revealed a confidence interval of 49 cM for the smallest detected QTL effect of 3.6 % explained phenotypic variance and a confidence interval of 11 cM for the greatest detected QTL effect of 16.9 %. In the case of small QTL the confidence interval span at least half of the linkage group, so that QTL locations could not exactly be separated.

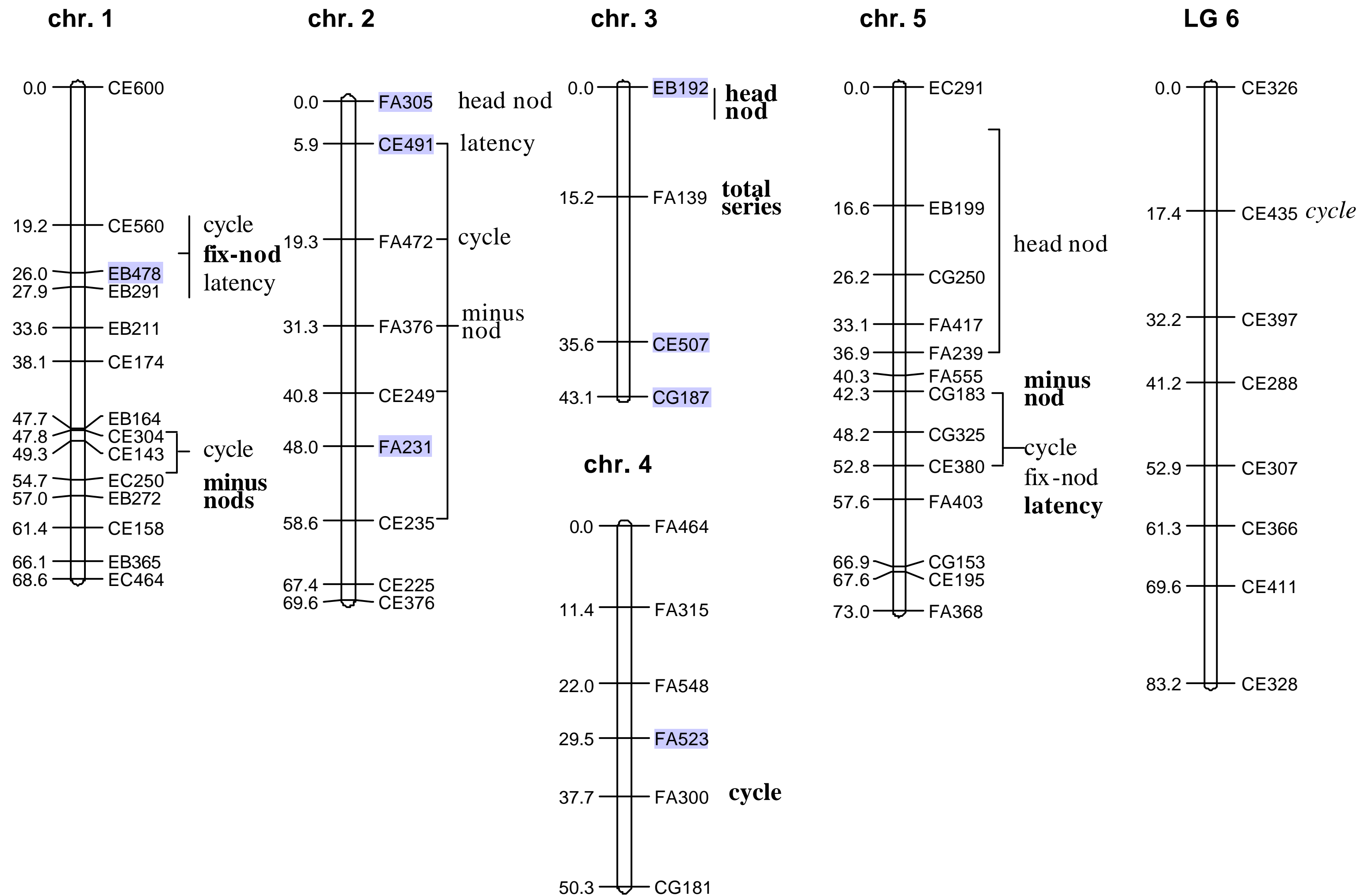

Figure 26: QTL for courtship components of an LV cross based on 298 individuals. The linkage map consists of 54 mapped AFLP markers based on a mapping population of 326 genotyped LV hybrid males. QTL marked in standard face have been previously detected on the respective chromsome in the initial QTL analysis (1st set). QTL marked in bold face have been detected for the first time. The QTL marked in italics could not be homologized on the chromosomal scale because the linkage group did not show a marker that could be assigned to a specific chromosome (see Figure 7, p. 35). Grey underlaid markers have been assigned to specific chromosomes.

Table 15

Significant courtship QTL based on a LV mapping population of 298 individuals and 54 mapped AFLP markers.

trait	total expl. var. [%]	LOD a <0.05[a]	chr.[b]	LOD / p*[2]	associated marker / interval	mean N. vit.	mean N. long.	expl. var. [%]	MQM - cofactor
cycle 1st	24.6	2.0	1	4.7	CE560, EB478	8.0	11.7	8.6	EB478
			1	2.7	CE143, EC250	8.8	11.8	4.2	EB478
			2	2.2	FA472	8.4	10.8	3.8	
			5	5.0	CE380	8.3	12.1	8.0	
cycle 2nd	15.4	2.4	1	3.3	CE304	9.2	11.5	5.4	CE304
			2	2.5	FA472	9.7	11.6	4.1	
			5	3.6	CG183	9.1	11.6	5.9	
cycle 3rd	19.0	2.5	1	3.7	EB478	9.0	11.3	7.9	
			5	5.1	CE380	8.9	11.7	11.1	
cycle 4th	38.1	2.4	?	3.0	CE435	9.0	10.8	7.2	
			1	3.0	EB478	9.4	11.5	7.0	
			1	2.4	CE143, EC250	9.5	11.7	6.9	
			5	7.7	CE380	9.2	12.4	17.0	
hnd 2nd	3.6	2.4	5	2.3	FA239	3.0	2.4	3.6	
hnd 3rd	4.2	2.4	3	2.8	EB192	3.3	2.8	4.2	
hnd 4th	11.0	2.3	2	4.0	FA305	3.5	2.7	6.2	
			3	2.3	EB192, FA139	3.4	2.8	4.8	
latency	17.8	2.4	1	3.4	CE560	125.5	189.9	7.7	
			2	3.1	CE491	123.7	175.5	5.5	
			5	2.6	FA403	132.1	184.2	4.6	
f1x 1stnod	14.2	2.1	5	3.4	CE380	4.6	8.5	6.4	
			1	3.5	CE560, EB478	4.3	8.3	7.8	
total series	5.6	2.3	3	2.3		14.1	10.4	5.6	
minus nods*[2]			1	0.0010*[2]	EC250				
			2	0.0000*[2]	EC491, FA376, FA472, CE249				
			5	0.0000*[2]	CE380				

[a] genome wide threshold

[b] chromosome number

*[1] QTL that has been already detected in the first QTL analysis (1st set)

*[2] Binary trait detected through linkage disequilibrium (Fisher's exact test)

QTL effect and the unit of reference

QTL effects can be measured in two different ways. In the first approach, a QTL effect is expressed as the proportion of the phenotypic variance occurring in the hybrid mapping population. The second measure refers to the variance occurring in the parental inbred strains (True et al. 1997). This variance is termed 'environmental variance' since inbred strains are supposed to show no genetic variance. This measure was used as a rough estimate of the phenotypic variance, since the analysis of wildtype courtship displays showed that the natural standing variation was in the same order of magnitude as the environmental variance of the inbred strains. The heritability in the broad sense (additive genetic variance and epistatic genetic variance) for courtship components ranged from 15 to 39 % (Table 16). These estimates were inferred through substracting the phenotypic variance of the inbred lines from the variance occurring in the wild populations removing the environmental variance component of the observed phenotypic variance in the wildpopulation.

Table 16
Variance of courtship components in a wildpopulation of *N. vitripennis* and in the inbred strains ASYMC.
(Sample size in brackets)

	variance wildtype	variance inbred strain	broad sense heritability[9]
head nod 1st	2.5 (188)	1.6 (93)	0.36
cycle 1st [sec]	3.3 (189)	2.8(68)	0.15
total number of series	7.0 (189)	4.3 (82)	0.39

Two different estimates of QTL effects for a selection of courtship and wing size traits for which parental phenotypes were available are shown in Table 17. Obviously, the ratio of the phenotypic variance between the parental strains and the LV hybrids differed profoundly between traits. For example, the variance for cycle time in the first cycle was more than twenty times higher in the LV hybrids than in the parental strains, whereas the variance for head nods in the first series were in the same order of magnitude between the hybrid and

9 Heritability in the broad sense estimates the genetic variance component of the total phenotypic variance occuring in a population. The genetic variance is composed of additive and epistatic genetic variance. Heritability in the narrow sense considers only the additive genetic variance.

parental strains. Therefore, QTL which explain a certain amount of the phenotypic variance differ in the magnitude of effect, when the QTL effect is referred to the environmental variance. For instance, a cycle time QTL which explains 12 % of the phenotypic variance in the hybrid population had an effect of the magnitude of the threefold environmental variance, whereas a head nod QTL explaining 21 % of the phenotypic variance had only an effect of 0.3 environmental variances. Interestingly, the morphological traits showed only a small effect in terms of environmental variance compared to most behavioural traits. In nearly all cases, the variance in the hybrid population is higher compared to the parental phenotypic variance except for seta density, where the variance is enhanced by one order of magnitude compared to the hybrid variance.

Table 17

Effects of significant QTL referred to the variance in the F_2 hybrid mapping population (explained variance [%]) or referred to the environmental variance of the parental inbred strains [environmental variance]

trait	environmental variance	variance LV hybrids	QTL effect explained variance [%]	QTL effect x times environmental variance[10]
cycle 1st	12.3	297.5	16.9	3.9
			13.7	3.3
			12.2	3.0
head nods 1st	4.1	5.0	21.0	0.3
total number of series	6.6	129.9	26.0	5.1
			23.9	4.7
			20.0	3.9
wing width (norm)	0.02	0.03	28.7	0.4
wing length x width (norm)	0.32	0.47	22.0	0.3
seta density	1043.2	365.1	16.0	0.1

Epistasis

We found several two way epistatic interactions for head nod, cycle and latency QTL among loci on different chromosomes with two modes of interaction. In one interaction the

10 A QTL effect of 3.9 times environmental variance has an effect that is 3.9 times larger than the variance occuring in the parentals.

conditional QTL led to an increased QTL effect at the primary QTL, whereas in the other mode the conditional allele led to a reversed allelic effect at the primary QTL. The reversed allelic effects at head nod QTL were affected by a *N. vitripennis* (V) allele at a conditional QTL (interaction A, B in Figure 27 and Table 18), whereas the *N. longicornis* (L) allele at the conditional QTL nearly neutralized the QTL effect at the primary QTL. Interestingly, the pure VV haplotype of interacting loci showed also a reversed effect (lowest number of head nods) implying additional higher order interactions.

Epistatic interactions detected for cycle time were exclusively conditioned upon L alleles (Figure 27, Table 18). In accordance with the parental phenotypes for cycle time the VV haplotypes of all epistatic interactions resulted in shorter cycle times than LL haplotypes. The epistatic interactions for latency (D, E) were conditioned upon L alleles and the recombinant LV haplotype showed the shortest latency.

Table 18
Epistatic interacting loci of courtship behaviour of LV hybrids (1st set)

Notation[a]	Trait	Lous 1	Locus 2	VV	VL	LV	LL	deviation from additivity	R²	LOD	adjusted p-value
A	head nods	A201080 (1)	Nv-23 (2)	1.7	4.7	3.5	2.8	2.3	0.09	20.8	0.00
B	head nods	A201080 (1)	Nv-33 (5)	1.8	5.0	3.3	3.0	2.9	0.13	14.1	0.00
C	total series	AD346 (1)	Nv-39 (2)	15.7	6.4	14.0	15.7	7.6	0.01	16.4	0.00
D	latency	Nv-24 (3)	AD346 (1)	117.2	122.5	80.7	228.8	142.8	0.14	6.1	0.00
E	latency	Nv-29 (3)	ED128 (2)	133.8	104.6	74.2	206.6	102.0	0.03	3.5	0.00
F	cycle time	AD519 (2)	Nv-45 (5)	9.3	12.3	9.9	30.5	17.6	0.10	14.6	0.03
G	cycle time	Nv-20 (2)	Nv-45 (5)	8.9	9.8	11.3	25.8	13.6	0.16	18.5	0.03
H	cycle time	Nv-25 (5)	Nv-40 (1)	5.9	14.2	11.8	28.3	14.1	0.24	11.4	0.00
I	cycle time	Nv-37 (3)	Nv-33 (5)	7.8	11.7	8.6	22.3	10.6	0.15	8.6	0.04

[a] The notation in the 1st column refers to the notation in the text and Figure 27. The following numbers in brackets behind locus names indicate the chromosome number. Columns VV, VL, LV, LL designate the phenotypic effects of respective haplotypes of locus 1 and 2. Shaded figures indicate the highest epistatic effect. R² (coeff. of determination). The LOD scores indicate the deviation from an additive interaction model. The adjusted p-value is the probability of obtaining a LOD score by chance (estimated by Monte-Carlo simulation and corrected for multiple tests (Materials and Methods).

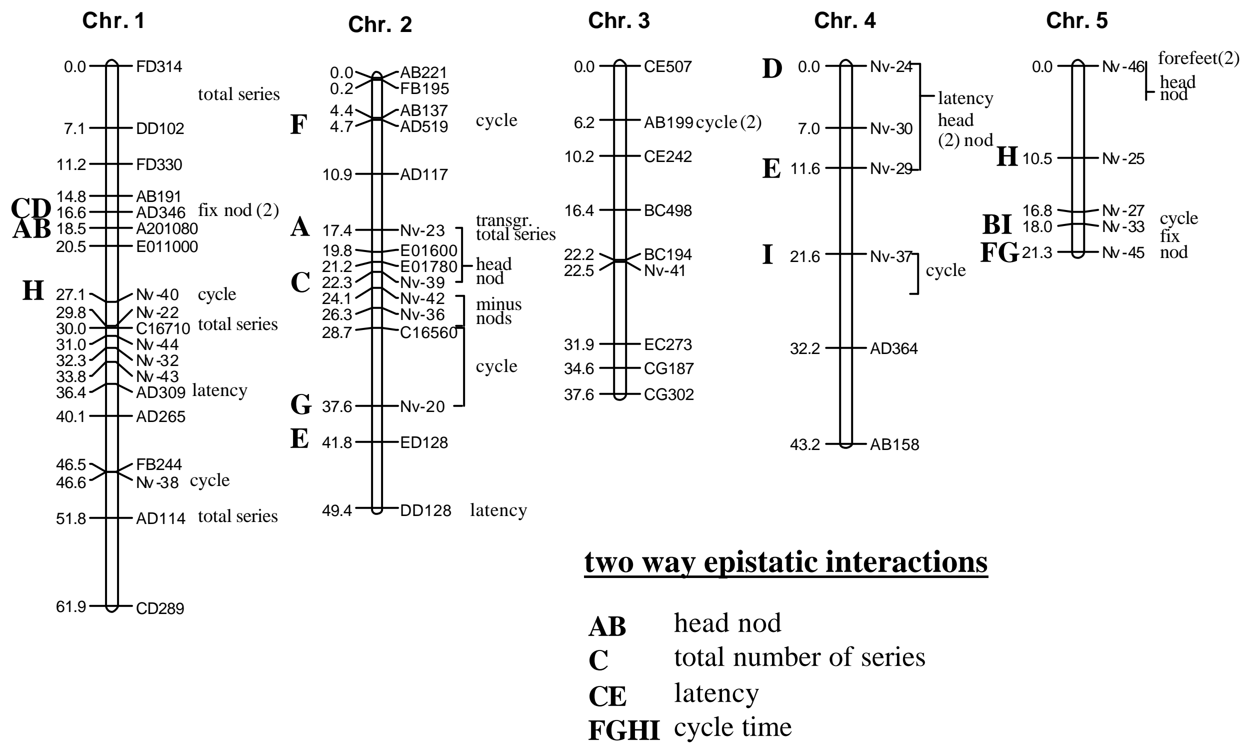

Figure 27: QTL detected in epistatic two-way interactions (capitals in bold face) and QTL detected in interval mapping (notation in standard face). For details see Table 18.

Discussion

One of the central question in evolutionary biology is how do new species come into existence and how do species evolve to become phenotypically distinct. The common basis of species concepts (reviewed in Coyne and Orr 2004) is that the process of speciation can be viewed as the genetic divergence of two populations to a point at which their subsequent merger would not be possible (Wu and Ting 2004). The Biological Species Concept (BSC) introduced by Dobzhansky (1935) and Mayr (1942) emphasized that the genetic independence of species result from the evolution of reproductive isolation (RI). The mechanisms of RI can be divided into those that act before fertilization (prezygotic) and those that proceed after fertilization (postzygotic). Prezygotic isolation prevent the formation of hybrids because the two species do not mate e.g. due to ecological or behavioural factors. In contrast, postzygotic isolation barriers such as hybrid sterility or inviability bar gene flow through hybrids. Both forms of reproductive isolation ensures that species remain genetically distinct, and consequently can undergo independent evolutionary fates (Orr and Presgrave 2000).

As a consequence, species can often be delineated by distinct phenotypes such as differences in courtship behaviour or morphological characters.

Genetics of behavioural an morphological species differences

Genetics of phenotypic species differences

The QTL anlyses of LV hybrids have shown that QTL for courtship behaviour were distributed over all chromosomes. QTL for cycle time head nods have been detected on four chromosomes, latency and minus nods on three chromosomes and fix-1st nod and for the total number of series on two different chromosomes. Only one QTL for forefeet rubbing have been mapped. This finding argues against a major pleiotropic effect exerting on mate

searching and courtship behaviour in *Nasonia*, although the difference in the grade of arousal between *N. vitripennis* and *N. longicornis* in the *N. vitripennis* behaviour, would speak for a major QTL influencing the entire behavioural sequence. This assumption comes from the finding that genes that influence complex behaviour often serve different functions (Hall 1994, Heisenberg 1997, Pflugfelder 1998, Sokolowski 2001). For instance, neurotransmitters and modlulators such as biogenic amines exert strong effects on a variety of sexual as well as non-sexual behaviours (Bicker and Menzel 1989, Yamamoto 1997). The sex specific transcript of the *fru* gene, however, appeared to expand its pleiotropic effects exclusively in the context of male sexual behaviour (Baker et al. 2001). The comparative analysis of male courtship displays within the genus *Nasonia* contradict the presence of a major pleiotropic effect in accordance with the QTL analyses because males of *N. giraulti* showed the longest cycle time, the highest number of head nods and an intermediate fix-1st nod within the genus (Assem and Werren 1994). This simple comparison highlights the need to study a phenomenon across several taxa when ever possible to gain additional support for an otherwhise propounded hypothesis (e.g. Roff 1995).

The spatial coincidence of QTL for cycle time, fix-1st nod and latency on chromosome one and five in the final QTL analysis based on 298 individuals may indicate a shared genetic basis for chronological courtship components. But the confidence intervals for QTL with approximately 10 % explained phenotypic variance spanned 18 cM. Thus, the resolution of the QTL analysis did not allow distinguishing the combined effects of closely linked QTL from a single pleiotropic locus. This problem could be tackeled with an increased marker density to detect recombination events within the interval containing the different QTL. Closely linked QTL could than be separated in the QTL analysis, when the sample size suffice to show a considerable recombination fraction between these loci. An appropriate approach to achieve the genetic dissection of closely QTL in *Nasonia* represents the experimental design of advanced intercross lines that are produced by randomly mated F_1

hybrids for t generations. The intercrossing for multiple generations leads to an increase of the recombination fraction betwee two loci proportional to the number of generations (Darvasi and Soller 1995). This approach allow for a higher resolution of QTL map position than do conventional F_2-designs, albeit of the expense of decreased power of QTL detection (Lynch and Walsh 1998, p. 432).

However, the analysis of epistatic interactions suggests a common genetic basis of QTL for head nods and the total number of series between chromsome 1 and 2 owing to the specific mode of interaction that led to a peculiar reversed allelic effect in the hybrid genome (Table 18).

The comparison of the five QTL analysis based on different sample size ranging from 94 to 298 individuals showed an overestimation of the QTL effects in experiments with low sample sizes. This so-called 'Beavis effect' has a simple theoretical basis. Given a normal distribution of error about a true QTL effect, errors involving overestimation surpass the significance threshold more often than those involving underestimation (Beavis 1994, Beavis 1998, Orr 2001) (see below). Therefore, the final QTL analysis based on 298 hybrids yielded the most accurate estimation of QTL effects in the study of courtship behaviour in the LV cross. Although, a set of QTL have not been recovered in the QTL analyses based on the AFLP map, probably due to differences in marker coverage compared to the 1st set analyis the final QTL analysis points towards a genetic architecture of many loci with small effects that govern phenotypic differences in courtship behaviour between *N. vitripennis* and *N. longicornis*. This conclusion comes from the fact that the total QTL effect of mapped QTL could explain only a fraction of the phenotypic variance occurring in the F_2 hybrid mapping population indicating that a large set of loci with small phenotypic effects remained undetected. Thus, the final QTL analysis sketched a genetic architecture that was different from the analyses based on 94 or 138 individuals, suggest the presence of QTL with large effects. The QTL model for cycle time in the second cycle explained up to 80 percent of the

phenotypic variance in the analysis of 94 hybrids, whereas the the inferred effect of mapped QTL in the final analysis fell below 20 percent. However, a set of unmapped markers indicate the existence of additional QTL that had not been mapped but may significantly increase the resolution of the entire QTL model (appendix #). For cycle time in the second cycle, for instance, unmapped markers accounted for 50 percent phenotypic variance in contrast to the QTL model of mapped markers that only explained 15 percent of the phenotypic variance in the final analysis. However, the superposition of phenotypic effects of unmapped markers that are actually physically linked may inflate their phenotypic effects to some degree.

Although the results provide little information about the actual distribution of QTL effects, they contradict Fisher's infinitesimal model of adaptive evolution. Fisher (1930) concluded that major mutations likely exert deleterious pleiotropic side effects in an n-dimensional phenotype and thus may disrupt the integrity of a complex organism.Therefore, Fisher's infinitesimal model assumed that micromutations produce the genetic variation on that selection can act upon. However, if this model would largely be correct, QTL analysis would be a hopless enterprise (Mackay 2001). Orr (1998) has shown that the phenotypic effects of mutations in the course of an adaptive walk follows an exponential distribution, i.e. the response to selection involves many factors of small effect and few factors of larger effect. The exponential model has been found to be robust to many assumptions and it appears to be a general property of adaptation to a fixed optimum (Orr 1998, 1999). Although the current resolution of the QTL analyses of courtship behaviour in *Nasonia* in the interspecific LV cross does not allow to draw inferences about the distribution of effects and congruence with Orr's exponential model it does not preclude its validity for the genetic differences in the courtship behaviour in *Nasonia*. Ambigiouties in locus order and insufficient marker density may provide an additional source of error that obscure the true distribution of phenotypic effects in the current analysis because estimation is profoundly influenced by the precision of the inference of QTL location (see below). The number of unmapped markers that were

associated with a significant phenotypic effect suggests that improved marker coverage may be the crucial factor to uncover additional factors to assess more accurate the distribution of phenotypic effects in the investigated LV cross.

However, the results of the QTL analyses of courtship behaviour do not rule out the existence of major QTL. There is, for instance, an indication for a major QTL (>10 % explained phenotypic variance according to Tanksley 1993) for cycle time on chromosome 5 that explained 8, 11 and 17 percent of the phenotypic variance in the first, second and fourth cycle, respectively. Furthermore, the phenotypic distribution of all courtship components showed a positive skewness, i.e. the tail to the right of the mean is longer than that to the left, indicating the presence of major genes, since skewed distributions may be effected by a mixture of distributions of different parameters (see Lynch and Walsh 1998 p. 353-364). Further indications come from *P*-element insertional mutations of large effects on *Drosophila* quantitative traits that affect a highly skewed and leptokurtic phenotypic distribution (Lyman et al. 1996). Therefore, the leptokurtosis (more peaked than expected from a given distribution) observed in the phenotypic distribution of courtship components gives an additional indication for the presence of major QTL in the male courtship behaviour in *Nasonia* (e.g. Figure 16, p. 48).

Normally, the magnitude of a QTL effect is referred to the phenotypic variance in the F_2 mapping population that is roughly equivalent to the phenotypic species gap. But from an evolutionary perspective, the phenotypic species gap might not represent the appropriate measure of a phenotypic effect, since selection is 'ignorant' to the species gap rather acting on the standing variation within species (Orr 2001). When QTL effects were measured relative to the variation that have been observed in the inbred strains ASYMC (*N. vitripennis)* and IV7R2 (*N. longicornis)* for courtship components and wing size measurements revealed some dramatic differences in the QTL effects. Wing size QTL, for instance, explained only a fraction of the environmental variance, whereas cycle time QTL have greater effects of

multiple environmental variances, although the effects of these QTL measured relative to the phenotypic variance in the F_2 population were quite similar (Table 15, p. 63). This might be affected by differences in the heritability between morphological and behavioural traits, since behavioural traits are assumed to show a larger environmental variance component of the phenotypic variance than morphological characters. Nethertheless, profound differences have also been found within behavioural traits, i.e. the environmental variance for head nods and the total number of series exhibited a similar magnitude, whereas QTL effects differed by one order of magnitude when they were measured relative to the environmental variance (Table 15, p. 63). The most rigorous approach would require the additional estimation of the heritability of a trait to infere the insights into the past evolutionary change between species. The estimation of the environmental variance of the parental inbred lines that were used for QTL analysis, however, will be the first step to assess the magnitude of a QTL effect in an evolutionary context.

The large body of QTL studies (reviewed in Mackay 2001 and Orr 2001) exhibit a great variety of the distribution and magnitude of effects that govern the phenotypic differences between species. It has even been shown, that species differences may result from few genes accounting for a large proportion of the phenotypic species difference (e.g. Edwards et al. 1987, Doebley 1992, Bradshaw et. al. 1998, Grandillo S. and S. D. Tanksley 1996, Gadau et al. 2002, Huttunen et al. 2004) or may even result from a single gene (Roelofs et al. 1987, Monti et al. 1997, Sucena and Stern 2000). But several studies also revealed a polygenic basis with many loci with small effects on the species differences, e.g. for male genital architecture between *Drosophila simulans* and *D. mauritiana* (Zeng et al. 2000), for differences in the puls rate in the male courtship song between *Laupala kohalensis* and *L. paranigra* (Shaw and Parsons 2002), and for trait differences in the mating system between *Mimulus guttatus* (large-flowered outcrosser) and *M. nasutus* (small-flowered selfer) (Fishman et al. 2002). Orr (2001) pointed out, that the data on species differences do not yet allow a deduction of a

consistent pattern of the genetic architecture of trait divergence. Indeed, differences may be simply affected by differences in population structure, strength of selection, and standing phenotypic variantion for each species pair. Furthermore, the phenotypic effect of QTL relative to the F_2 variance in the mapping population will change with time, i.e. the first mutation will explain hundred percent of the species gap, whereas consecutive mutations diminish the relative effect of the first mutation. Therefore, this estimate declines with divergence time, whereas the estimation of QTL effect relative to the standing phenotypic variance within populations is assumed to be roughly time invariant (Orr 2001). Again, the estimated effects of QTL for courtship behaviour and wing size differences in the *Nasonia* cross between *N. vitripennis* and *N. longicornis* have shown that QTL effects can easily be misinterpreted, when estimated relative to the F_2 variance. Therefore, I would recommend, that QTL studies intended to address questions concerning the evolution of trait differences should additionally take the phenotypic variance of parental strains (as a rough estimate) or the heritability of a trait into account to estimate the magnitude of QTL effects.

The significance of courtship behvaviour

When *Nasonia* species are cured from *Wolbachia* infections, they were able to interbreed, thus prezygotic isolation is not complete under lab conditions, although asymmetric sexual isolation occurs between species (Bordenstein et al. 2000, Beukeboom and Assem 2001, Bordenstein et al. 2001). Assem et al. (1980) have shown that the pheromone discharge at the first head nod of each series during the courtship display serves as the predominant stimulus to elicit the female's receptivity. So fare, there are no experimental evidence for the role of the courtship display in mate discrimination in *Nasonia*.

Jachmann and Assem (1996) proposed that the performance of the highly ritualised courtship display is a self-inhibitory process in which head nodding play the key role. With every head not a cumulative inhibition is build up, which brings the display to an end.

Especially the first head nod series have a predictive property on the total number of series. Head nodding is not necessary for successful induction of the female's receptivity, since males, which had been prevented to perform head nods were equal successful in mating as fully intact males (Assem 1986).

The epistatic interaction between chromosome 1 and 2 for head nods and the total number of series provide the genetic connection for the negative relation between head nods and the number of series in the courtship display (see Table 18, p. 66). In contrast to *N. longicornis* that exclusively parasitize fly pupae in bird nests, *N. vitripennis* also parasitize fly pupae on carcass, likely encountering a higher male-male competition for mates since larger host clutch sizes will lead to a balanced sex ratio, whereas the sex ratio on small clutch sizes may be more female biases (local mate competition. see Werren 1983). Therefore, *N. vitripennis* males may encounter a stronger selective pressure to reduce time investment spent on a single mating rather than exert more effort in securing multiple mates (Parker 1974). The pronounced wing size differences in *N. vitripennis* may give an additional indication for a stronger selective pressure due to increased male-male competition in *N. vitripennis* than in *N. longicornis* because it has been shown in wingdimorphic insects, that the micropterus morphs develop faster and hence reproduce earlier than the macropterus morphs (Roff 1995). This fits in the observation, although not systematically investigated, that *N. vitripennis* develops faster than *N. longicornis* and *N. giraulti*, respecitivelly. Male *N. vitripennis* wait at their emergence site to mate their sisters to follow. Faster development will increase fitness, since males in *N. vitripennis* mate at their emrgence site and defend territories at the emergence hole monopolizing emerging females when males are present at low density. But when the male density increases, territoriality breaks down and males scramble for emerging females (Assem et al. 1980a).

Recombinant genome and phenotype

In hybrids two genomes have to interact, that never have been tested by natural selection before. This may lead to the break up of coadapted gene complexes and is assumed to cause a suite of hybrid incompatibilities (see above). Another feature occurring in hybrids referred to as transgressive phenotypes is that individual hybrids may show more extreme character values than are seen in either parental species (Rieseberg et al 1999, Rieseberg et al. 2000). Non-additive interlocus interactions referred to as epistasis and cosegregation of complementary genes have often been put forward to explain transgressive segregation in hybrid populations (see RIESEBERG ET AL. 1999, 2003). Complementary genes are alleles reducing a trait that derive from the species with the higher mean character value and vise versa. Stochastic combination of 'high' alleles that derive from both parental strains may give rise to hybrid individuals with transgressive phenotypes.

A variety of hybrid effects shaping the phenotypic distribution in the interspecific hybrid crosses between *N. longicornis* and *N. vitripennis*. The large proportion of hybrid males with a high number of series led to a transgression of the average phenotypes beyond the parental extremes. The detected QTL on chromosome 1 with a complementary gene action could not account alone for the excess of transgressive phenotypes on a population wide scale. The cosegregation of 'high' alleles can only explain a transgressive phenotype in individual hybrids. In fact, the epistatic interaction with a locus in direct vicinity to the ‘transgressive’ QTL for the number of series reversed the QTL effect, i.e. hybrids with a *N. vitripennis* allele at all head nod QTL behaved more *N. longicornis* like and vice versa. The effect was even present in hybrids with a pure *N. vitripennis* haplotype of both interacting QTL (Table 18). Though higher order interactions with heterospecific loci are supposed to be involved in that hybrid specific effect.

Extremely prolonged courtship displays far beyond the parental phenotypic ranges are obviously a hybrid dysfunction. They have been interpreted as a malfunction in feedback

loops controlling courtship duration in hybrid males by Jachmann and Assem (1996). Single individuals in hybrid males perform extraordinary prolonged courtship displays that give the impression that these males go on and on courting an unreceptive female without giving up.

The reversal of allelic effects was additionally observed for head nod QTL. The negative correlation between the mean number of head nods and the proportion of *N. vitripennis* genome indicates that the strength of the reversal effect on the individual phenotype appears to be dependent on the proportion of *N. vitripennis* genome. Thus, undetected head nod QTL that can explain a large proportion of phenotypic variance should react in the same direction. A reversed sign of an allelic effect of QTL for anal plate bristle number and sex comb tooth number has already been described in reciprocal crosses of *Drosophila simulans* and *D. mauritiana* by True et al. (1997). This effect was also accounted to backcross specific effects on the QTL effect.

The conspicuous mode of espistatic interactions shared by QTL for head nods and the total number of series and the spatial coincidence argue for a shared genetic basis (Table 18, interaction A, B and I). This epistatic interaction provides the connection between the performance of head nods and the inhibition of courtship as proposed by Jachmann and Assem (1996). This interlocus interaction can be assumed to provide a functional link between head nods and the total number of series required for the inhibitory model from Jachmann and Assem (1996). Therefore, it is supposed to exist in the parental species as well to trigger the time investment in a single courtship display. The reversal effect is likely evoked by heterospecific interactions.

The detection of a bias towards the phenotype of the grandparental species in the 480 phenotyped LV hybrids for most courtship components corroborates the findings of Beukeboom and Assem (2001), who described the so-called 'grandfather effect' in both reciprocal crosses. In the following, I will concentrate on the number of head nods and the total number of series because binary traits and latency have been quantified on a categorical

scales in the parental strains, and therefore were not amenable to a statistical anaylsis on an interval scale. Furthermore, the trait fix-nod has not been considered by Beukeboom and Assem (2001).

Epistasis plays a role in altering the allelic action for the number of head nods and the total number of series. The combined effect of biased recovery due to hybrid incompatibilites and epistasis will therefore lead to a shifted phenotype towards the grandparental species. Furthermore, a hybrid dysfunction may also contribute to the 'grandfahter effect' for the total number of series, since the mean value exceeds the N. longicornis phenotype that showed the highest number of series in a courtship display within the *N. vitripennis* - *N. longicornis* species pair.

To infer the contribution of nucleo-cytoplasmic interactions to the ‘grandfather effect’ Beukeboom and Assem (2002) constructed *N. vitripennis* and *N. longicornis* hybrids with the ‘grand paternal genome’ residing in its conspecific cytoplasm to circumvent nucleo-cytoplasmic incompabibilites (Beukeboom and Assem 2002). The ‘grandfather effect’ could be recovered, though in a weaker tendency, indicating some nuclear remnants of the maternal donor in the introgression lines. In the same study an introgression lines which was assumed to bear complete a *N. vitripennis* nuclear genome in a *N. longicornis* cytoplasm behaved almost like the pure *N. longicornis* line for the number of head nods. This provides indication for either the presence of introgression barriers of paternally derived nuclear genes in the maternal cytotyp or the impossibility to break up of specific co-adapted nuclear genes in a recombinant genetic background due to epistatic interactions (e.g. Chun-Xiao et al. 2000). Comparing the segregation distortion of shared markers in the LV and VL cross indicate the presence of introgression barriers of paternal genes due to the cytoplasmic background. Taking the reversal of allelic effects into account, the biased recovery rate of N. vitripennis genome was likely involved in the 'grandfather effect'. Hybridization experiments in multiple artificial and natural occurring sunflower hybrids indicated that genes may be not randomly

inherited in hybrids to prevent the break up of coadapted gene complexes (Rieseberg et al. 1996).

Hybrid incompatibility

Intrinsic hybrid incompatibility

The analysis of haplotypes in the reciprocal hybrid crosses revealed an asymmetry in abdundance and complexity of incompatibilities between the LV and VL cross. Most obvious, in the VL cross on third more incompatibilities have been detected than in the LV cross. This finding corresponds to a stronger F_2-hybrid breakdown associated with the *N. longicornis* cytoplasm observed in reciprocal crosses (pers. comm. L. Beukeboom). Pairs of markers that show a significant excess of maternal haplotypes in both crosses (maternal alleles corresponding to the cytoplasm) differed between the VL and LV cross. The increase in maternal haplotype frequencies were affected in the *N. longicornis* cytoplasmic background in the VL cross by nuclear-nuclear as well as nuclear-cytoplasmic incompatibilities. The excess of maternal haplotype frequencies in the LV cross resulted in most cases solely from nuclear-cytoplasmic incompatibilities. The more complex situation in the VL cross cannot be explained exclusively on the higher rate of nuclear-nuclear incompatibilities assuming an equal mutation rate in both species. The more frequent nuclear-nuclear incompatibility in the VL cross compared to the LV cross calls for negative higher order interactions between sets of nuclear genes and cytoplasmic factors (nuclear-nuclear-cytoplasmic incompatibilities). Indeed, there is evidence, that the cytotyp of the sister taxa group of *N. longicornis* and *N. giraulti* has a strong influence on the formation of more complex incompatibilities with the nuclear genome of *N. vitripennis*. The same asymmetry of hybrid incompatibilities has been found between the reciprocal crosses of *N. vitripennis* and *N. giraulti* (Breeuwer and Werren 1995). Negative interactions of *N. vitripennis* nuclear genes with the *N. giraulti* cytoplasm are involved in severe F_2-hybrid breakdown, whereas incompatibilities were much weaker in the

reciprocal cross. Even the introgression of *N. vitripennis* nuclear genes in the *N. giraulti* cytoplasm for five generations did not overcome a constant high rate of mortality indicating a specific retention of *N. giraulti* nuclear genes providing an introgression[11] barrier of the *N. vitripennis* nuclear genome. Moreover, the *N. giraulti* phenotype persisted to some extent, although the *N. giraulti* genome should have been displaced to ~ 95 %. Hybrid incompatibilities have only been observed in crosses between *N. vitripennis* and the representatives of the sister taxa *N. longicornis* and *N. giraulti* (phylogenetic relations see Campbell et al. 1993), whereas hybrid incompatibilities within the sister group were nearly absent (Bordenstein et al. 2001). Therefore, it can be assumed that the *N. vitripennis* cytoplasm represent a more ancestral state, that retained crucial functional compatibilities with the genome of *N. longicornis* and *N. giraulti* group. This consideration is based on the prediction of the Dobzhansky-Muller model that derived genotypes are more likely to be involved in hybrid incompatibilities than ancestral alleles. Simply spoken, derived alleles could be incompatible with either ancestral or other derived alleles, whereas ancestral alleles could only be incompatible with a derived allele. According to the Dobzhansky-Muller model, derived alleles are three times more likely to be involved in incompatibilities than are ancestral alleles (Orr 1995). Muller (1942) mentioned that hybrid incompatibilities should be asymmetric, considering that *B* in Figure 28 might be incompatible with *A*, but *a* is completely compatible with *b* (see also Coyne and Orr 2004).

[11] Introgression barriers are genes that prevent the recovery of heterospecific alleles in the hybrids due to hybrid inviability.

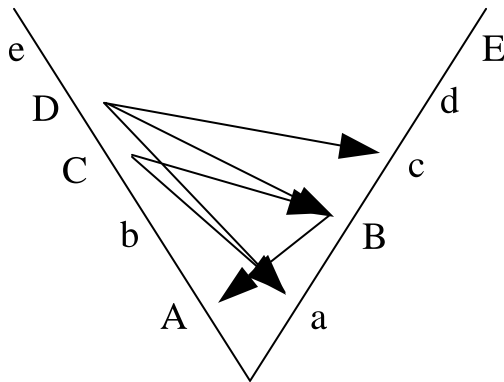

Figure 28: The evolution of genic incompatibilities between two allopatric populations. Time runs upward. The first substitution occurs at locus **a**, the second at the **b** locus. Ancestral alleles are delineated in lower case and derived alleles in upper case. Possible genic incompatibilities in hybrids are shown as arrows. (From Orr 1995)

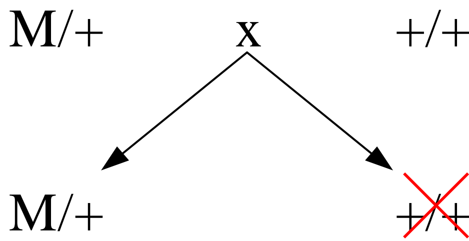

Figure 29: Maternal effect allele M as a cytoplasmic factor lead to death of progeny of a heterozygous mother that did not inherit the M allele. (From Hurst and Werren 2001)

The analysis of haplotype frequencies of loci that were involved in hybrid inviability siggest that cytoplasmic factors are involved in the observed asymmetry in hybrid incompatibilities in *Nasonia*, but it has not been established yet that the cytoplasmic factor involved in hybrid breakdown in *Nasonia* belongs to the mitochondrial genome (Breeuwer and Werren 1995). Maternal-effect genes, for instance, cannot be ruled out to be involved in hybrid-breakdown asymmetries. Maternal effect genes like the *Medea* locus (maternal-effect dominant embryonic arrest) in *Tribolium castaneum*, for instance, kills the progeny that do not inherit the maternal effect allele of a heterozygous mother (Beeman et al. 1992) (Figure 29). This maternal effect gene act as a post-segregation distorter promoting its own spread in the population (Hurst and Werren 2001) and might therefore involved in a biased segregation ratio of linked loci. Maternal effect genes that cause cause embryonic-lethality have also be found in mammals (Hurst 1993) and in *Drosophila* (Sawamura et al. 1993 a,b).

Gadau et al (1999) suggested that maternal-effect genes might be a factor explaining the overall bias towards the maternal *N. vitripennis* genome in a F_2 mapping population between an *N. giraulti* introgression line (R16A) and the *N. vitripennis* line (ASYMC). In the introgression line the *N. vitripennis* nuclear genome is supposed to be completely exchanged by *N. giraulti* nuclear genes through consecutive backcrossing to the *N. giraulti* strain for ten

generations. Therefore, hybrid incompatibilities caused by negative nucleo-cytoplasmic interactions in the F_2 generation have been excluded to explain the bias towards maternal alleles in the hybrid mapping population. However, the abundance of hybrid incompatibilities detected in *N. vitripennis* x *N. longicornis* crosses and their dispersion over all chromosomes do not support the hypothesis of a monogenic maternal effect gene causing a biased recovery rate of maternal alleles.

Introgression experiments between *N. vitripennis* and *N. giraulti* (Breeuwer and Werren 1995) and *N. vitripennis* and *N. longicornis* (Beukeboom and Assem 2002), however indicate the retention of maternal alleles in *Nasonia* hybrids because hybrids still showed maternal behaviour after multiple generations of backcrossing. Therefore it is likely that the observed biased recovery rate of maternal alleles could be at least partially caused by retained maternal factors in the introgression lines. The same phenomenon has been observed in LV and VL lines. Beukeboom and Assem (2002) introgressed the parental nuclear genome in the heterospecific cytotyp in reciprocal crosses between *N. vitripennis* and *N. longicornis* through backcrossing with males of the parental line for ten generations. The phenotypic analysis of courtship behaviour revealed significant differences between the pure line and the introgresssed line for cycle time and the number of head nods and additionally in the other introgression line for minus nods and forefeet rubbing. This finding indicates an incomplete replacement of the maternal genome in the introgression lines. The introgression barriers were found to be asymmetric between the reciprocal introgression lines, e.g. the introgression of heterospecific genes appeared to be more restricted in the *N. longicornis* cytotype due to a more severe F_2-hybrid breakdown (pers. comm. L. Beukeboom) corresponding to the more complex hybrid incompatibilities found in the VL cross. Hower, the mapping of hybrid incompatibilities on the linkage group in both reciprocal crosses, revealed that loci that are involved in hybrid incompatibilities are distributed over all chromsomes indicating complex nuclear-nuclear as well as nuclera-cytoplasmic interactions rather than an oligogenic basis of

hybrid inviability involving one or severarl maternal factors. However, this finding does not preclude the existence of maternal factors.

Behavioural sterility

The affliction of hybrid behaviour results from two different forms of hybrid incompatibilities. In the intrinsic form of hybrid incompatibility hybrids fail to reproduce because they suffer a neurological, or pheromonal, etc. defect that renders them incapable of effective courtship, although they might be fertile. In the extrinsic form, hybrids display intermediate behaviours or pheromones, etc. that do not fit the females preference (Coyne and Orr 2004). Under the experimental paradigm that was used to observe courtship behaviour of LV hybrid males, mostly intrinsic driven hybrid dysfunctions in the mate searching and courtship behaviour have been assessed qualitatively, i.e. the reduced locomotor acitivity and disruptions in consecutive steps of mate searching and courtship behaviour (see Figure 19, p. 51). The influence of intermediate behaviour, as an extrinsic hybrid incompatibility, of copulation success was not evaluated since already mated females were used.

The most frequent behavioural dysfunction was a reduced locomotor activity of hybrids resulting in a failure to court within five minutes. Similarly, reduced locomotor acitivity and reduced courtship intensity[12] has been observed in F_1-hybrid males of reciprocal crosses between *Drosophila pseudoobscura* and *D. persimilis* (Noor 1996). The behavioural dysfunctions were asymmetric between the reciprocal crosses, and could be attributed to an interaction between the X-chromosome of *D. persimilis* and the autosomal Y-chromosome from *D. pseudoobscura* (Noor 1997). Haldane was the first who noticed that a reduced fitness in hybrids is preferentially found in the heterogametic sex (Haldane 1922). This observation known as Haldane's rule has been confirmed in many cases of hybrid crosses (reviewed in

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[12] Courtship intensity is defined as the ratio of the time period to the first courtship and the time the male spent performing courtship (Noor 1996).

Craft 1983, Coyne and Orr 1989, Coyne 1992, Wu and Davis, Laurie 1997, Presgraves 2002, Price and Bouvier 2002, Tubaro and Lijtmaer 2002, Lijtmaer et al. 2003).

Owing to the haploidy in hymenopteran males, *Nasonia* males may be more susceptible to hybrid incompatibilities such as sterility, inviability than diploids, since negative epistatic interactions between autosomal loci can be instantaneously phenotypically expressed. Indeed, the advantage of the haplo-diploid genetics in *Nasonia* has been used to screen the entire genome for recessive mutations affecting developmental processes (Pultz et al. 1999, 2000, Pultz and Leaf 2003). Behavioural dysfunctions have never been reported yet in females and were only found in F_2 hybrid males. For instance, VG[13] males were unable to court and suffered from dysfunctions in locomotor ability whereas males of the reciprocal GV cross mated and sired offspring (Breeuwer and Werren 1995). LV-hybrid males, for instance showed less courtship success in an thirty minutes assy than the reciropcal VL cross and the final copulation success after twenty four hours was only eighty percent of that of the VL hybrids (Beukeboom and Assem 2001). Thus, the strength of behavioural sterility occurring in LV hybrids compared to VL hybrids is not congruent with the asymmetry in F_2-hybrid breakdown mapped in both reciprocal crosses.

The impairment of locomotor activity in hybrids may intail a fitness loss in male-male competition. Male-male competition will be more severe when the male frequency increases in the mating pool especially in gregarious parasitoid species that parasitize patchy distributed hosts as it is the case in *Nasonia,* and males tend to stay at the emergence site due to the high predicatability of emerging females (Godfray 1994, Godfray and Cook 1997). Additionally, prolonged hybrid courtship displays may also contribute to a hybrid disadvantage since *Nasonia* males are unable to distinguish mated from virgin females hence reducing the

[13] Courtship intensity is defined as the ratio of the time period to the first courtship and the time the male spent performing courtship (Noor 1996)

frequency of potential matings (Assem 1986). Thus, courtship behaviour may contribute to postzygotic isolation although the divergence might have been driven by intrasexual selection.

Therefore, behavioural dysfunctions in locomotor activity and courtship in *Nasonia* may serve as a postzygotic isolation barrier and appear to be a behavioural manifestation of Haldane's rule. There is growing evidence that sexual selection contributes to hybrid sterility in males through a faster divergence of genens involved in reproduction than in females due to intra- as well as intesexual selection (Wu and Davis 1993, Wu et al. 1996, True et al. 1996, Hollocher and Wu 1996 Presgrave and Orr 1998, Tao et al. 2003, Tao and Hartl 2003).

Altough all three *Nasonia* species are reproductively isolated through *Wolbachia* endobacteria, this isolation is not complete (Werren 1997) and it is unclear whether *Wolbachia* infections preceeded the evolution of other reproductive isolation barriers in *Nasonia* (but see Bordenstein et al. 2001). However, the significance of mate searching and courtship behaviour as reproductive isolating factors can only properly assessed by evaluating life history traits in nature concerning e.g. asymmetries in female mate discrimination, developmental time, and abundance of males and females in host patches.

Technical comments

Dominant versus codominant markers

The mapping efficiency of dominant markers in the LV, VL and LV_VL maps did not reach thirty percent except for the final LV map based on 326 hybrid males in contrast to the codominant microsatellite markers that showed a mapping efficiency of seventy percent. Since dominant AFLP markers were equally informative in the haploid F_2 cross design (equivalent to a backcross design), the lower mapping efficiency could not be attributed to a lower linkage information content that occurs for dominant markers in a diploid F_2 cross design (Liu 1998. p. 193-198). The susceptibility of dominant markers to genotyping errors, (alleles are scored as present or absent) may have contributed to the low mapping efficiency.

Another crucial factor in the multilocus PCR reaction of AFLP markers might be the superposition of bands of equal size that refer to different loci, imposing an additional possibility of genotyping errors (Beukeboom pers. comm.). AFLP's however do allow constructing linkage maps with wide genome coverage without expense of marker development. Additionally, one AFLP-amplification yields multiple loci in one reaction though genotyping is much faster than in standard single locus PCR reaction of codominant markers. Weighing the pros and cons, I would plead for a combination of dominant and codominant markers in QTL analysis especially in non-model organisms, for that a coherent sequence information is not yet available. AFLP's or RAPD's can be used to achieve the genome coverage and codominant markers as microsatellites serve as anchor points within the linkage groups.

Marker distribution and genome coverage

The number of unmapped QTL that had been detected with increasing sample size indicates that the genome coverage with markers was not completely represented in the inferred linkage maps. These genome fractions that are not covered by the linkage map will be excluded in subsequent QTL analysis and therefore will affect the power and resolution of QTL detection. Incomplete marker coverage may result from a small number of markers, non-random marker distribution, small sample size, or thight linkage grouping criteria (Liu 1998, p. 345-349). A low number of markers can be excluded to affect marker coveage in the linkage map construction in the reciprocal *Nasonia* crosses, but there are indications for the non-random distributions of microsatellite markers that tend to cluster in the central region of the linkage groups (see Figure 22, p. 56). Further, it is likely that the sample size of more than 300 individuals did not suffice to resolve five linkage groups that represent the five chromosomes because a large linkage group of 83 cM could not be attached to an existing linkage group that had been already assigned to a specific chromsome. Since the number of

genotyped markers were high, such additional linkage groups may result from a to small sample size (Liu 1998, p. 348). Linkage grouping thresholds of a LOD score 15 with a recombination threshold of 0.35 were used to resolve at least five linkage groups. Relaxed grouping thresholds, however, led to an amalgamation of several linkage groups into a single one. However, the susceptibility of dominant markers to genotyping errors (see above) can not be excluded to contribute to a reduced resolution of the linkage map.

Sample size

Sample size matter in several respect in inferential statistics for QTL identification. The influence on sample size of the estimation of QTL effects has been demonstrated in QTL analyses with increasing sample size (see Figure 24, p. 60). This estimation bias should be diminished to only slight overestimations when a sample size of 500 individuals will be evaluated (Beavis 1994, 1998). The overestimation of QTL effects result from the statistical power to detect a QTL. If the sample size is low, the power to detect QTL of small effect is small. Hence if such a QTL will be detected by chance, the effect will be overestimated (Broman 2001, Xu 2003). An increased sample size incraeses the power for QTL detection, leading to a more accurate estimate of QTL with moderate or large effects, but the bias associated with newly detected QTL with small effects remains worse (Xu 2003). Therefore, I would advise for a careful interpretation of the distribution of phenotypic effects when the sample size is below 500 individuals especially when behavioural traits with low heritabilities are investigated due to a reduction in statistical power.

Further, an increased sample size will help to break up closely linked QTL since only the fraction of the mapping population which shows recombination between closely linked QTL will be informative for the inference of multiple closely linked QTL.

Summary

The genetics of species differences is an outstanding question in evolutionary biology. How do species evolve to become phenotypically distinct and how is the genetic architecture organized that underlie species differences? Phenotypic diverged traits are supposed to be frequently involved in prezygotic isolation, i.e. they prevent the formation of hybrids, whereas postzygotic isolation occurs when hybrids experience a fitness reduction.

The parasitic wasp genus *Nasonia* represents an appropriate model system to investigate the genetics of species differences as well as the genetics of postzygotic isolation. The genus consists of three species *N. vitripennis*, *N. longicornis* and *N. giraulti* that differ particularly in male traits that are assumed to posses an adaptive significance: courtship behaviour and wing size differences. The courtship behaviour consists of cyclically repeated series of head nods that are separated by pauses. The stereotypic performance allowed to split up the display into distinct courtship components. Males of *N. vitripennis* bear vestigial forewings and are incapable of flight, whereas *N. longicornis* wear intermediate sized wings and *N. giraulti* is fully capable of flying.

Nasonia species can produce interspecific hybrids after removing *Wolbachia* bacteria induced hybrid incompatibilities with antibiotics. Postzygotic isolation occurs to different extent and is asymmetric among reciprocal crosses, e.g. inviability is stronger in the *N. vitripennis* (?) x *N. longicornis* (?) cross than in the *N. longicornis* (?) x *N. vitripennis* (?) cross.

The formation of hybrids allow to study the genetic of species differences in QTL (quantitative trait locus) analyses as well as the genetics of postzygotic isolation causing hybrid inviability.

The aim of the study was to investigate the genetic architecture of differences in courtship behaviour and wing size between *N. vitripennis* and *N. longicornis* and to assess the

genetics of postzygotic isolation to gain clues about the evolutionary processes underlying trait divergence and establishment of reproductive isolation between taxa.

In a QTL analysis based on 94 F_2-hybrid individuals of an LV[14] cross only few QTL for wing size differences have been found with relatively large effects, although a large proportion of the phenotypic variance remained unexplained. The QTL on courtship behaviour analysis based on 94-F_2 hybrid males revealed a complex genetic architecture of courtship behaviour with QTL of large phenotypic effects that explained more than 40 % of the phenotypic variance in one case. Additionally, an epistatic analysis (non-additive interlocus interaction) of courtship QTL revealed frequent genetic interchromsomal relations leading in some instances to hybrid specific effects, e.g. reversion of phenotypic effects or the transgression[15] of phenotypes. A QTL analysis based on a threefold sample size revealed, however, an overestimation of QTL effects in the analysis based on smaller sample size pointing towards a genetic architecture of many loci with small effects governing the phenotypic differences in courtship behaviour.

Furthermore, the the study comprised the analysis of postzygotic isolation in the reciprocal crosses *N. vitripennis* (?) x *N. longicornis* (?) versus *N. longicornis* (?) x *N. vitripennis* (?) located several loci distributed over different chromosomes that are involved in hybrid incompatibility. The mapping of hybrid incompatibility regions reproduced for the first time the observed asymmetries in the strength of postzygotic isolation in reciprocal crosses of between the more distant related taxa within the genus *Nasonia*. Stronger postzygotic incompatibilities in the VL cross are supposed to result from the superposition of nuclear-nuclear incompatibilities with nuclear-cytoplasmic incompatibilities, whereas the coincidences of these to types of incompatibilities were found to be much weaker in the reciprocal LV cross.

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[14] L stands for *N. longicornis* and V for *N. vitripennis*. Hybrid crosses are designated by the paternal genotype followed by the maternal genotype.
[15] Hybrid phenotypes that fall beyond the phenotypical range of the parental strains.

Zuammenfassung

Die Genetik von Artunterschieden ist einer der herausragenden Fragen der Evolutionsbiologie. Auf welche Weise entwickeln sich Arten phänotypisch auseinander? Divergierende Merkmale sind häufig an präzygoter Isolation beteilgt, das heißt, die Verhinderung von Hybridpaarungen, wohingegen die postzygote Isolation auftritt, wenn Hybride einen Fitnessverlust erleiden.

Die parasitische Wespengattung *Nasonia* stellt ein hervorragendes Modellsystem zur Untersuchung der Genetik von Artunterschieden und der Genetik von postzygoter Isolation dar. Die Gattung besteht aus den drei Arten *N. vitripennis*, *N. longicornis* und *N. giraulti*, die sich in wahrscheinlich adaptiven Merkmalen der Männchen unterscheiden: Paarungsverhalten und Unterschiede in der Vorderflügelgröße. Das Paarungsverhalten besteht aus wiederkehrenden Kopfstoßserien, die durch Pausen getrennt sind. Aufgrund der stereotypen Ausbildung kann das Paarungsverhalten in distinkte Verhaltenskomponenten aufgeteilt werden. Männchen von *N. vitripennis* weisen verkleinerte Vorderflügel auf und sind flugunfähig. *N. longicornis* weist intermediäre Flügel auf und Männchen von *N. giraulti* besitzen voll ausgebildete Flügel und sind flugfähig.

Nasonia Arten sind in der Lage interspezifische Hybride zu bilden, unter vorheriger Eliminierung von *Wolbachia* induzierten Hybridinkompatibilitäten durch Behandlung mit Antibiotika. Postzygote Isolation tritt in unterschiedlichem Ausmaß auf und ist asymmetrisch zwischen reziproken Kreuzungen, z.B. ist die Lebensunfähigkeit von Hybriden stärker in der Kreuzung *N. vitripennis* (?) x *N. longicornis* (?) als in der reziproken Kreuzung *N. longicornis* (?) x *N. vitripennis* (?) ausgeprägt.

Die Bildung von interspezifischen Hybriden erlaubt die Untersuchung der genetischen Architektur von Artunterschieden in der QTL (quantitative trait locus) Analyse als auch die Untersuchung der postzygoten Isolation, die Hybridzusammenbruch verursacht.

Das Ziel dieser Arbeit war es, die Genetik von Unterschieden im Paarungsverhalten und Flügelgröße zwischen *N. vitripennis* und *N. longicornis* und die Genetik der postzygoten Isolation zu untersuchen, um Hinweise auf die evolutionären Prozesse zu gewinnen, die zur Divergenz von Artunterschieden und zur Etablierung von reproduktiver Isolation geführt haben.

In einer QTL Analyse, die auf einer Kartierungspopulation von 94 F_2-Hybriden einer LV[16] Kreuzung basierte, konnten nur wenige QTL für Unterschiede in der Flügelgröße detektiert werden, die jedoch einen großen phänotypischen Effekt aufwiesen. Die QTL Analyse, die ebenfalls auf 94 LV-Hybriden basierte, ergab eine komplexe genetische Architektur des Paarungsverhaltens mit QTL, die einem Fall über 40 % der phänotypischen Varianz erklären konnten. Zusätzlich ergab eine Analyse von epistatischen (nichtadditive Interaktion zwischen Loci) viele interchromosomale Ineraktionen, die in einigen Fällen zu Hybrideffekten wie die Umkehrung von allelischen Effekten oder Transgression[17] von Phänotypen führten. Eine QTL Analyse, die auf einer dreifachen Stichprobengröße beruhte, deutete allerdings auf eine starke Überschätzung phänotypischer Effekte bei der QTL Analyse mit kleinerer Stichprobe hin. Vielmehr scheinen die Unterschiede im Paarungsverhalten auf einer genetischen Architektur von vielen Faktoren mit kleineren phänotypischen Effekten zu beruhen.

Die Analyse der postzygoten Isolation in den reziproken Kreuzungen *N. vitripennis* (?) x *N. longicornis* (?) und *N. longicornis* (?) x *N. vitripennis* (?) lokalisierte eine Reihe von Loci auf verschiedenen Chromosome, die am Hybridzusammenbruch beteiligt sind. Mit der Kartierung von Incompatibilitätsloci konnten zum ersten Mal die beobachtete Asymmetrie der postzygoten Isolation zwischen weiter entferntverwandten Taxa in der Gattung *Nasonia* genetisch nachvollzogen werden. Die stärker ausgeprägte Hybridinkompatibilität in der VL

[16] L steht für *N. longicornis* und V für *N. vitripennis*. Hybride werden durch den Genotyp der väterlichen Linie und anschließend durch den Genotyp der maternalen Linie bezeichnet.
[17] Phänotypen von Hybriden, die sich außerhalb des phänotypischen Bereichs der partentalen Linien befinden.

Kreuzung scheint von der Überlagerung von nukleär-nukleärer Inkompatibilität und nukleär-zytoplasmatischer Inkompatibilität zu resulieren. Die Überlagerung dieser beiden beiden Formen der Hybridinkompatibilität ist in der reziproken LV Kreuzung wesentlich schwächer ausgeprägt.

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Appendix

appendix 1

Microsatellite DNA markers that are polymorphic between the inbred strains ASYMCHS (*N. vitripennis*) and IV7R2 (*N. longicornis*). Ta: annealing temperature, N.V.: *N. vitripennis* allele, N.L.: *N. longicornis* allele.

Locus	Ta [°C]	Primer sequence (5'-3')	Repeat motif	N.V [bp]	N.L. [bp]	GenBank accession no.
N1-14	52.0	CGC TGT TTG TAT AAG TTG AAA GTC G CCC CGA GCA AAA GTG TCG	$(AT)_6(bp)_{108}(TC)_3(bp)_{12}(TC)_3$	231	-	
N1-15	56.0	ATT TTC TCG GCG GTA AAA CC GTG GGG GAC CAT AAT GAA CG	$(GT)_4GCGG(GT)_3$	183	-	
N1-26	52.0	TTG ACG GAG AAG TTC CAG TGG ATA TCT GCA GAA TTC G	$(TG)_9$			
N1-28	53.0	TGA GCT AGA GAC GAT ACG AAC G CTA TTC GCC TTT TTG CTT CC	$(TG)_{21}$	201	-	
N1-29	58.0	TGC GGA ACA TAT CAA ACT CG AGC GAC ATG CCC AAG TAG G	$(TG)_6G(TG)$	235	-	
N1-30	58.0	ATG GTG GGT TTT TCT TTT CG AAA GAA CAC GCA ACA AAC TCC	$(AT)_{31}(GT)_8$	228	-	
N1-31	56.0	TCC GCC GAA TTA ACT AAT GG GAG GGT GGA GAA GGA AAA GG	$(TC)_{16}$	190	-	
N1-41	50.0	CAA TCC CAA ACG AAT TTT CC CAA CTG CCT TTG CCA TCC	$(TG)_8C(GT)_5ATGT(TA)_{21}$	250	-	
N1-45	58.0	CAA GTT CAT TCG AGC AAT GG ACA TAT CAC GGT ATA ATA CAC ACA CC	$(TG)C(TG)_{10}$	221	-	
N1-46	52.0	AAA AAG GAT CGT CGA TTT GG AGA TCT CGT TGG CTT TTT CG	$(TG)_{18}$	247	-	
N1-5	57.0	GCC CTT GTA GAC TGC GTA CC GAA GAG TCT CCC GAA AAA GG	$(CG)_4CCG(GC)$	177	-	
NB-15	50.0	ATT TTC TCG GCG GTA AAA CC GTG GGG GAC CAT AAT GAA CG	$(GT)_4GCGG(GT)_3$	183	-	
NB-2	52.0	TCA CGT GTT TTT AGA AAA TGT CC GGG AAT TGG TTT TTG TTA AAC G	$(GT)_{11}$	271	-	
NB-7	48.0	CTC GAT AGG ATT ATG CC CTA ATT TCC CTA CAG ACC	$(TG)_9TA(TG)_2$	116		
Nv-17	47.0	AAG AAT GTA TCA AGT ATG AGC C TCA GTT CTT GAA ACG TTG C	$(GT)_{13}$	215	227	AY262049
Nv-18	52.0	CTA TTC ATC TTC ACT CAC ATC G CAG GAG AAT GAG ACT TGA GC	$(TG)_4GG(TG)_5$	234	228	AY262042
Nv-19	52.0	TTA CTG CCG AAT AAT ACC C GCT TTG GCG TTA TAA TCC	$(TG)_8TT(TG)_6$	186	170	AY262043
Nv-20	55.0	TGA CGA AGT ATC CGA GAA G TCG AAA AAC GAT ATT GCT CG	$(GT)_{12}$	105	89	AY262039

Locus	Ta [°C]	Primer sequence (5'-3')	Repeat motif	N.V [bp]	N.L. [bp]	GenBank accession no.
NV-21	47.0	TTG ATA TTG ATC GAG TAG TTC C CTC CTA GAG GAA GCT TTG C	$(TG)_9TA(TG)_2$	177	165	AY262040
NV-22	54:0	ATT TCA CCG CGC TAT AAC AGC TAG GGA AGA GGA TAC C	$(GA)_{17}(GT)_{10}$	202	220	AY262041
NV-23	50.0	CAG CAT ACT CAA GCA AGC GAT ACC TGA AGT TTG ATG C	$(GT)_{11}AT(GT)_5(AT)_6GT(AT)_4$	217	-	AY262044
NV-24	50.0	CCG AAA TCC ACA TAG ACC AGG AAC TCA TCA AGA CGG	$(GT)_{15}$	116	100	AY262045
NV-25	52.0	ATA TTG ACG GAG TAG TTC C GGC CTT CTT ACA TTA TAC C	$(GT)_5GACG(TG)_9$	200	-	
NV-26	52.0	TTC GCA GCT TTC CTT TGC AGC AGC TAG TAT GAA CCG	$(GT)_{20}$	142	120	AY262047
NV-27	52.0	AAT ACT CGC TGT TCA ATC G CGC TAG ATC GGA TTT CCG	$(GT)_8(GC)_2(GT)_6$	193	169	AY262048
NV-29	48.0	TTG ACG GAG TAG TTC GCA TGG CTA CTC CGA ATT TCC	$(GT)_9$	266	-	
NV-30	50.0	TCC CGG GAA TAT CAA AGC GCG AGT ATT CTC GTT TGG	$(TG)_8$	154	-	
NV-31	52.0	TCT CTT GAT TAG GTC ATG C GTG ACC ATA AAT GTC TAT GC	$(TG)_{16}G(TG)$	104	117	-
NV-32	50.0	TAC TTC CGA GTT TCA TTA TTG G ACA ACG GAA ACC CAA AGC	$(TG)_8$	115	111	
NV-33	52.0	TGT TAA ATT TTA TTG ATA TTG ACG TGT GTT AGA AGT TCA AAT CG	$(TG)_5(CG)_2(TG)_7$	140	133	
NV-34	48.0	GTC GAA TTC CTT CTG G TAA TGT TCT TTG TGA CC	$(TG)_{11}$	149	134	
NV-35	50.0	CTA TTG TTT GTT ATC GC CTG GTG TCT ACA CTG C	$(TG)_8$	170	171	
NV-36	52.0	TCG ATC CAG ATG AAG AGG AGA GAA TTA AGA GAA AGT CCG	$(GT)_{21}$	185	170	
NV-37	48.0	TAG GAC CGT GAA GTT TGC TGT TAC TGC TCT GGA ACG	$(AT)_6C(TG)_{12}$	236	-	
NV-38	52.0	GGT ATT GAC CAA GTA TTT CC CTC CTA GAG GAA GCT TTG C	$(TG)_{11}TA(TG)_6$	196	-	
NV-39	53.0	GTA GTT GCA GAA ATA TTA CG ATG CGG TCT CTT AAT AGG	$(GT)_7$	214	-	
NV-40	48.0	TAT CGA GAG AAA GAA GGT AGG ATT AAG TAA CGC TTG CTC G	$(TG)_{12}$	228	219	
NV-41	52.0	GTC AGA CGT GGG CTT TGT C TTA TGC GCC ACA CAC ACC	$(GT)_{15}T(GT)_3$	251	233	

Locus	Ta [°C]	Primer sequence (5'-3')	Repeat motif	N.V [bp]	N.L. [bp]	GenBank accession no.
NV-42	55.0	CTC TGT TGC TCG ACG TAC C GGA TTT AGA GGT GTT GTG TCC	$(TG)_{28}$	271	-	-
NV-43	56.0	TTC TCT AAC TTG CTC GCC GGA GTT GTG ACT TGT GAG C	$(TG)_9(AT)_{19}$	273		
NV-44	50,0	ATC GAT TAT TCC TAC ACC TG ATG GCC AAT AGT TCA CAT C	$(TG)_{10}C(GT)_5$	289	286	-
NV-45	55.0	AGA GTA TTA TGA GCC GAC TGG TCT AGC GCT CGT TCT TAC G	$(TG)_9CGCG(TG)_6A(TG)$	297	-	
NV-46	52.0	TTA CGT CAA GGT ATA GCT GC AAT AAG TGG CTG AAA GTT CC	$(TG)_{11}C(TG)_9$	300	297	

appendix 2

Primer sets for the pre-selective and selective PCR for AFLP generation.

primere	**code*[1]**	**concen-tration**	**sequence 5'-3'* preselective primer**
Eco-A		20µM	GACTGCGTACCAATTC**A**
Eco-C		20µM	GACTGCGTACCAATTC**C**
Eco-G		20µM	GACTGCGTACCAATTC**G**
Eco-T		20µM	GACTGCGTACCAATTC**T**
Mse-C		20µM	GATGAGTCCTGAGTAA**C**

name	**code**		**sequence selective primer**	**fluorescent label**
Eco-AAC	**A**	1 µM	GACTGCGTACCAATTC**AAC**	NED
Eco-AAG	**B**	1 µM	GACTGCGTACCAATTC**AAG**	HEX
Eco-ACA	**C**	1 µM	GACTGCGTACCAATTC**ACA**	FAM
Eco-ACC	**D**	1 µM	GACTGCGTACCAATTC**ACC**	NED
Eco-ACG	**E**	1 µM	GACTGCGTACCAATTC**ACG**	HEX
Eco-ACT	**F**	1 µM	GACTGCGTACCAATTC**ACT**	FAM
Eco-AGC	**G**	1 µM	GACTGCGTACCAATTC**AGC**	NED
Eco-AGG	**H**	1 µM	GACTGCGTACCAATTC**AGG**	HEX
Eco-CAA	**I**	1 µM	GACTGCGTACCAATTC**CAA**	HEX
Eco-CCT	**J**	1 µM	GACTGCGTACCAATTC**CCT**	FAM
Eco-GGA	**K**	1 µM	GACTGCGTACCAATTC**GGA**	HEX
Eco-GTT	**L**	1 µM	GACTGCGTACCAATTC**GTT**	FAM
Eco-TAG	**M**	1 µM	GACTGCGTACCAATTC**TAG**	FAM
Eco-TGC	**N**	1 µM	GACTGCGTACCAATTC**TGC**	HEX
Mse-CAA	**A**	17µM	GATGAGTCCTGAGTAA**CAA**	
Mse-CAC	**B**	17µM	GATGAGTCCTGAGTAA**CAC**	
Mse-CAG	**C**	17µM	GATGAGTCCTGAGTAA**CAG**	
Mse-CAT	**D**	17µM	GATGAGTCCTGAGTAA**CAT**	
Mse-CTA	**E**	17µM	GATGAGTCCTGAGTAA**CTA**	
Mse-CTC	**F**	17µM	GATGAGTCCTGAGTAA**CTC**	
Mse-CTG	**G**	17µM	GATGAGTCCTGAGTAA**CTG**	
Mse-CTT	**H**	17µM	GATGAGTCCTGAGTAA**CTT**	

* Selective nucleotides, that were added to the specific primer sequence are marked in bold face.
*[1] The coding of selective primers are used to identiy AFLP loci with the code of the selective *EcoRI* primer followed by the selective *MseI* primer and the fragment lenght.

appendix 3

Segregation ratio of shared markers between the LV and VL cross
V: N. vitripennis allele, L: N. longicornis allele, -: missing data,
* 0.05, ** 0.01, *** 0.005, **** 0.001, etc

LV cross

Locus	V	L	-	X2	Signif.
BC194	49	40	5	0,9	-
BC309	51	38	5	1,9	-
BC407	54	35	5	4,1	**
BC498	54	35	5	4,1	**
CE242	53	36	5	3,3	*
CE266	54	35	5	4,1	**
CE268	50	39	5	1,4	-
CE328	42	47	5	0,3	-
CE491	54	35	5	4,1	**
CE507	59	30	5	9,4	****
CG187	45	43	6	0,1	-
CG191	54	34	6	4,5	**
CG250	43	45	6	0,1	-
CG255	53	35	6	3,7	*
CG302	43	45	6	0,1	-
CG395	65	23	6	20	*******
CG434	55	33	6	5,5	**
EB118	77	12	5	48	*******
EB192	54	35	5	4,1	**
EB198	45	44	5	0	-
EB226	66	23	5	21	*******
EB383	59	30	5	9,4	****
EB391	68	21	5	25	*******
EB422	60	29	5	11	****
EB478	33	56	5	5,9	**
EB500	56	33	5	5,9	**
EC273	46	43	5	0,1	-
EC276	44	45	5	0	-
EC295	53	36	5	3,3	*
EC432	59	30	5	9,4	****
EC560	57	32	5	7	***

VL cross

Locus	V	L	-	X2	Signif.
BC194	35	44	3	1	-
BC309	36	43	3	0,6	-
BC407	25	54	3	11	****
BC498	32	47	3	2,9	*
CE242	28	51	3	6,7	***
CE266	12	67	3	38	*******
CE268	11	68	3	41	*******
CE328	39	40	3	0	-
CE491	27	52	3	7,9	****
CE507	26	53	3	9,2	****
CG187	16	63	3	28	*******
CG191	59	20	3	19	*******
CG250	68	11	3	41	*******
CG255	5	74	3	60	*******
CG302	66	13	3	36	*******
CG395	14	65	3	33	*******
CG434	15	64	3	30	*******
EB118	34	45	3	1,5	-
EB192	20	59	3	19	*******
EB198	26	53	3	9,2	****
EB226	32	47	3	2,9	*
EB383	19	60	3	21	*******
EB391	20	59	3	19	*******
EB422	32	47	3	2,9	*
EB478	47	32	3	2,9	*
EB500	29	50	3	5,6	**
EC273	61	18	3	23	*******
EC276	17	62	3	26	*******
EC295	12	67	3	38	*******
EC432	31	48	3	3,7	*
EC560	14	65	3	33	*******

Locus	V	L	-	X2	Signif.	Locus	V	L	-	X2	Signif.
FA231	44	35	15	1	-	FA231	30	49	3	4,6	**
FA305	43	36	15	0,6	-	FA305	38	41	3	0,1	-
FA340	46	33	15	2,1	-	FA340	28	51	3	6,7	***
FA417	37	42	15	0,3	-	FA417	38	41	3	0,1	-
FA472	54	25	15	11	****	FA472	34	45	3	1,5	-
FA523	49	30	15	4,6	**	FA523	38	41	3	0,1	-
Nv-22	56	37	1	3,9	**	NV-22	41	41	0	0	-
Nv-27	40	19	35	7,5	***	NV-27	39	39	4	0	-
Nv-41	52	36	6	2,9	*	NV-41	33	48	1	2,8	*
Nv-44	55	35	4	4,4	**	NV-44	30	46	6	3,4	*
Nv-46	53	37	4	2,8	*	NV-46	26	54	2	9,8	****

appendix 4

Segregation ratio of shared markers between the LV and VL cross

V: N. vitripennis allele, L: N. longicornis allele, -: missing data,

* 0.05, ** 0.01, *** 0.005, **** 0.001, etc

locus	*N. vitripennis* allele	*N. longicornis* allele	missing data	chi²	Signif.
CE141	201	100	25	33.9	*******
CE143	227	74	25	77.8	*******
CE158	257	44	25	150.7	*******
CE170	227	74	25	77.8	*******
CE174	222	79	25	67.9	*******
CE178	279	22	25	219.4	*******
CE182	153	148	25	0.1	-
CE188	257	44	25	150.7	*******
CE195	193	108	25	24.0	*******
CE207	227	74	25	77.8	*******
CE210	96	205	25	39.5	*******
CE215	95	206	25	40.9	*******
CE221	198	103	25	30.0	*******
CE225	145	156	25	0.4	-
CE235	197	104	25	28.7	*******
CE242	221	80	25	66.0	*******
CE249	181	120	25	12.4	******
CE260	100	201	25	33.9	*******
CE266	127	174	25	7.3	***
CE268	130	171	25	5.6	**
CE271	224	77	25	71.8	*******
CE288	205	96	25	39.5	*******
CE304	230	71	25	84.0	*******
CE307	222	79	25	67.9	*******
CE314	201	100	25	33.9	*******
CE317	188	113	25	18.7	*******
CE321	172	129	25	6.1	**
CE326	199	102	25	31.3	*******
CE328	187	114	25	17.7	*******
CE335	88	213	25	51.9	*******
CE366	238	63	25	101.7	*******
CE369	234	67	25	92.7	*******
CE376	173	128	25	6.7	***
CE380	223	78	25	69.8	*******
CE387	148	153	25	0.1	-
CE396	165	136	25	2.8	*
CE397	171	130	25	5.6	**
CE411	213	88	25	51.9	*******
CE435	146	155	25	0.3	-
CE475	94	207	25	42.4	*******
CE491	161	140	25	1.5	-
CE507	243	58	25	113.7	*******
CE560	159	104	63	11.5	*****
CE584	126	137	63	0.5	-
CE600	122	141	63	1.4	-
CG102	144	150	32	0.1	-
CG105	165	129	32	4.4	**
CG117	63	231	32	96.0	*******
CG130	131	163	32	3.5	*
CG140	231	63	32	96.0	*******
CG141	205	89	32	45.8	*******
CG146	75	219	32	70.5	*******
CG153	181	113	32	15.7	*******
CG155	150	144	32	0.1	-
CG157	156	138	32	1.1	-
CG161	123	171	32	7.8	***
CG166	138	156	32	1.1	-
CG181	158	136	32	1.6	-
CG183	214	80	32	61.1	*******
CG185	143	151	32	0.2	-
CG187	245	49	32	130.7	*******
CG191	120	174	32	9.9	****
CG192	212	82	32	57.5	*******
CG198	128	166	32	4.9	**
CG201	121	173	32	9.2	****
CG205	179	115	32	13.9	******
CG207	176	118	32	11.4	*****
CG209	215	79	32	62.9	*******
CG213	209	85	32	52.3	*******
CG215	82	212	32	57.5	*******
CG225	236	58	32	107.8	*******
CG250	214	80	32	61.1	*******
CG255	144	150	32	0.1	-

locus	*N. vitripennis* allele	*N. longicornis* allele	missing data	chi²	Signif.
CG267	172	122	32	8.5	****
CG291	235	59	32	105.4	*******
CG302	226	68	32	84.9	*******
CG325	200	94	32	38.2	*******
CG348	222	72	32	76.5	*******
CG361	83	65	178	2.2	-
CG395	205	89	32	45.8	*******
CG434	171	123	32	7.8	***
CG506	131	126	69	0.1	-
CG549	166	51	109	60.9	*******
CG89	80	214	32	61.1	*******
EB115	256	67	3	110.6	*******
EB118	94	229	3	56.4	*******
EB120	68	255	3	108.3	*******
EB125	238	85	3	72.5	*******
EB126	119	204	3	22.4	*******
EB132	96	227	3	53.1	*******
EB153	248	75	3	92.7	*******
EB156	107	216	3	36.8	*******
EB164	214	109	3	34.1	*******
EB174	252	71	3	101.4	*******
EB178	252	71	3	101.4	*******
EB181	241	82	3	78.3	*******
EB188	261	62	3	122.6	*******
EB192	192	131	3	11.5	*****
EB194	283	40	3	182.8	*******
EB198	93	230	3	58.1	*******
EB199	184	139	3	6.3	**
EB207	121	202	3	20.3	*******
EB210	135	188	3	8.7	****
EB211	222	101	3	45.3	*******
EB226	106	217	3	38.1	*******
EB246	138	185	3	6.8	***
EB257	261	62	3	122.6	*******
EB267	205	118	3	23.4	*******
EB272	267	56	3	137.8	*******
EB284	187	136	3	8.1	****
EB285	177	146	3	3.0	*
EB290	177	146	3	3.0	*
EB291	236	87	3	68.7	*******
EB359	133	190	3	10.1	****
EB362	134	189	3	9.4	****
EB365	220	103	3	42.4	*******
EB383	113	210	3	29.1	*******
EB387	283	40	3	182.8	*******
EB391	108	215	3	35.5	*******
EB422	109	214	3	34.1	*******
EB478	226	97	3	51.5	*******
EB482	110	213	3	32.9	*******
EB500	161	162	3	0.0	-
EB568	166	124	36	6.1	**
EB577	197	93	36	37.3	*******
EB86	29	294	3	217.4	*******
EC105	222	94	10	51.9	*******
EC109	141	175	10	3.7	*
EC112	217	99	10	44.1	*******
EC113	191	125	10	13.8	******
EC120	146	170	10	1.8	-
EC123	157	159	10	0.0	-
EC132	155	161	10	0.1	-
EC155	29	287	10	210.7	*******
EC171	291	25	10	223.9	*******
EC176	119	197	10	19.3	*******
EC192	259	57	10	129.1	*******
EC196	238	78	10	81.0	*******
EC199	242	74	10	89.3	*******
EC201	187	129	10	10.7	****
EC205	100	216	10	42.6	*******
EC214	305	11	10	273.5	*******
EC216	212	104	10	36.9	*******
EC218	149	167	10	1.0	-
EC231	178	138	10	5.1	**
EC236	260	56	10	131.7	*******
EC250	235	81	10	75.0	*******
EC268	101	215	10	41.1	*******
EC273	270	46	10	158.8	*******
EC276	82	234	10	73.1	*******
EC288	252	64	10	111.8	*******
EC291	148	168	10	1.3	-
EC295	34	282	10	194.6	*******
EC298	124	192	10	14.6	******

locus	*N. vitripennis* allele	*N. longicornis* allele	missing data	chi²	Signif.
EC302	111	205	10	28.0	*******
EC324	160	156	10	0.1	-
EC327	151	165	10	0.6	-
EC349	158	158	10	0.0	-
EC382	105	211	10	35.6	*******
EC384	122	194	10	16.4	*******
EC392	173	143	10	2.9	*
EC403	242	74	10	89.3	*******
EC407	111	205	10	28.0	*******
EC432	133	183	10	7.9	****
EC464	243	73	10	91.5	*******
EC484	148	168	10	1.3	-
EC495	128	188	10	11.4	*****
EC560	144	172	10	2.5	-
EC83	241	75	10	87.2	*******
EC86	154	162	10	0.2	-
FA110	84	214	28	56.7	*******
FA125	124	174	28	8.4	****
FA130	106	192	28	24.8	*******
FA139	172	126	28	7.1	***
FA164	246	52	28	126.3	*******
FA182	252	46	28	142.4	*******
FA192	204	94	28	40.6	*******
FA195	157	141	28	0.9	-
FA201	253	45	28	145.2	*******
FA213	208	90	28	46.7	*******
FA218	206	92	28	43.6	*******
FA231	165	133	28	3.4	*
FA235	210	88	28	50.0	*******
FA239	235	63	28	99.3	*******
FA247	175	123	28	9.1	****
FA258	204	94	28	40.6	*******
FA263	186	112	28	18.4	*******
FA277	257	41	28	156.6	*******
FA289	153	145	28	0.2	-
FA300	208	90	28	46.7	*******
FA305	161	137	28	1.9	-
FA315	180	118	28	12.9	******
FA326	268	30	28	190.1	*******
FA340	178	120	28	11.3	*****
FA355	230	68	28	88.1	*******
FA368	193	105	28	26.0	*******
FA376	187	111	28	19.4	*******
FA403	215	83	28	58.5	*******
FA417	212	86	28	53.3	*******
FA427	191	107	28	23.7	*******
FA464	189	109	28	21.5	*******
FA472	191	107	28	23.7	*******
FA523	176	92	58	26.3	*******
FA548	171	97	58	20.4	*******
FA555	186	82	58	40.4	*******
FA610	164	105	57	12.9	******

appendix 5

Linkage disequilibirium (LD) between mapped loci in the reciprocal crosses LV and VL

a adjustment for 213 significant LD (a =0.05) in the LV cross (a*=0.00024) and for 626 significnat LD in the VL cross (a*=0.00003)

LV cross

Locus 1	Locus 2	Chi	p-value	Chrom Loc 1	Chrom Loc 2
E011002	Nv-39	21,64	0	1	2
E011001	E01780	18,631	0	1	2
E011000	E01600	16,094	0	1	2
AD519	E011000	20,253	0	2	1
AD117	E011001	15,915	0	2	1
A201081	Nv-24	16,755	0	1	4
A201080	AD364	18,867	0	1	4
AD309	AD364	18,23	0	1	4
AB191	AD364	infinity	highly significant	1	4
AD364	C16710	15,326	0	4	1
Nv-25	Nv-38	16,67	0	5	1
Nv-26	Nv-39	15,973	0	5	2
Nv-42	Nv-46	17,359	0	2	5
Nv-39	Nv-47	infinity	highly significant	2	5
CE507	Nv-37	18,23	0	3	4
CG302	Nv-38	18,867	0	3	4
Nv-37	Nv-41	infinity	highly significant	4	3

VL cross

Locus 1	Locus 2	chi	p	Chrom Loc 1	Chrom Loc 2
EB120	EB157	infinity	highly significant	1	2
EB120	EB362	15,751	0	1	2
EB120	EB383	15,804	0	1	2
EB289	EB362	17,61	0	1	2

EB289	EB391	21,64	0	1	2
EB157	EB200	15,283	0	2	1
EB157	EB289	infinity	highly significant	2	1
EB157	EB483	19,807	0	2	1
EB391	EB422	16,361	0	2	1
EB391	EB483	16,291	0	2	1
BC309	BC194	17,61	0	1	3
BC309	BC498	infinity	highly significant	1	3
BC309	BC515	infinity	highly significant	1	3
BC309	CE328	infinity	highly significant	1	3
BC309	CE507	infinity	highly significant	1	3
BC309	EB500	infinity	highly significant	1	3
BC309	EC485	infinity	highly significant	1	3
BC309	Nv-41	infinity	highly significant	1	3
BC309	FA213	16,937	0	1	5
EB347	Nv-46	infinity	highly significant	1	5
EB478	FA213	19,807	0	1	5
BC144	BC216	21,64	0	5	1
BC144	EB347	15,859	0	5	1
EB178	EB347	15,599	0	5	1
EB178	EB478	16,755	0	5	1
FA213	FA239	20,253	0	5	1
FA213	FA417	20,253	0	5	1
EB226	EB347	infinity	highly significant	4	1
EB226	EB347	infinity	highly significant	4	1
FA187	FA472	18,631	0	2	4
CE215	EB352	infinity	highly significant	4	2
CE215	EB391	17,359	0	4	2
FA231	Nv-46	15,699	0	4	5

appendix 6

Shared significant LD in the reciprocal crosses.

VL				LV			
Locus 1	Locus 2	chi	p-value	Locus 1	Locus 2	chi²	p-value
100	445	21,64	0.0000	100	445	infin	0.0000
100	577	infin	0.0000	100	577	infin	0.0000
117	400	16,937	0.0000	117	400	infin	0.0000
117	419	infin	0.0000	117	419	infin	0.0000
117	542	infin	0.0000	117	542	infin	0.0000
117	562	infin	0.0000	117	562	infin	0.0000
117	591	16,033	0.0000	117	591	infin	0.0000
122	338	infin	0.0000	122	338	23,026	0.0000
122	350	infin	0.0000	122	350	infin	0.0000
122	513	17,748	0.0000	122	513	infin	0.0000
130	313	19,134	0.0000	130	313	infin	0.0000
130	445	infin	0.0000	130	445	infin	0.0000
130	577	19,807	0.0000	130	577	infin	0.0000
151	445	infin	0.0000	151	445	20,253	0.0000
151	577	21,64	0.0000	151	577	infin	0.0000
163	331	infin	0.0000	163	331	infin	0.0000
163	526	infin	0.0000	163	526	infin	0.0000
163	531	21,64	0.0000	163	531	infin	0.0000
163	552	infin	0.0000	163	552	19,442	0.0000
182	313	infin	0.0000	182	313	infin	0.0000
182	322	18,23	0.0000	182	322	18,421	0.0000
182	445	infin	0.0000	182	445	infin	0.0000
182	577	infin	0.0000	182	577	infin	0.0000
200	445	18,23	0.0000	200	445	infin	0.0000
251	463	infin	0.0000	251	463	21,64	0.0000
251	535	infin	0.0000	251	535	19,442	0.0000
256	400	infin	0.0000	256	400	infin	0.0000
256	591	23,026	0.0000	256	591	infin	0.0000
313	445	infin	0.0000	313	445	infin	0.0000
322	445	20,253	0.0000	322	445	23,026	0.0000
331	526	infin	0.0000	331	526	18,421	0.0000
338	557	23,026	0.0000	338	557	infin	0.0000
350	500	18,867	0.0000	350	500	infin	0.0000
350	513	15,699	0.0000	350	513	infin	0.0000
400	542	infin	0.0000	400	542	infin	0.0000
400	562	infin	0.0000	400	562	infin	0.0000

Locus 1	Locus 2	chi	p-value	Locus 1	Locus 2	chi²	p-value
400	591	infin	0.0000	400	591	infin	0.0000
419	542	infin	0.0000	419	542	23,026	0.0000
419	562	infin	0.0000	419	562	infin	0.0000
419	591	16,67	0.0000	419	591	infin	0.0000
431	313	16,51	0.0000	431	313	infin	0.0000
431	577	infin	0.0000	431	577	infin	0.0000
445	577	infin	0.0000	445	577	infin	0.0000
535	200a	18,867	0.0000	535	200a	20,253	0.0000

Locus notation contains chromosome number and position within the chromosome in cM (e.g. 445: marker on chromosome 4 at 45 cM)

infin: infinity

appendix 7

Contingeny talbles of shared significant LD between marker pairs in both reciprocal crosses.

Marker names are composed of chromosome number and position within the chromosome in cM (e.g. marker 445 is located on chromosome 4 on position 45 cM).

VL	**LV**
Pop: 1, Loci: 445 and 100 445 1 2 100 1.1 20 27 47 2.2 8 24 32 28 51 79	Pop: 1, Loci: 445 and 100 445 1 2 100 1.1 19 11 30 2.2 27 22 49 46 33 79
Pop:1, Loci: 577 and 100 577 1 2 100 1.1 35 12 47 2.2 26 6 32 61 18 79	Pop:1, Loci: 577 and 100 577 1 2 100 1.1 19 14 33 2.2 27 29 56 46 43 89
Pop:1, Loci: 400 and 117 400 1 2 117 1.1 17 19 36 2.2 17 26 43 34 45 79	Pop:1, Loci: 400 and 117 400 1 2 117 1.1 44 7 51 2.2 33 5 38 77 12 89
Pop: 1, Loci: 419 and 117 419 1 2 117 1.1 14 21 35 2.2 11 31 42 25 52 77	Pop: 1, Loci: 419 and 117 419 1 2 117 1.1 33 17 50 2.2 20 15 35 53 32 85

Pop:1, Loci: 542 and 117 542 1 2 117 __________ 1.1 18 17 35 2.2 13 30 43 __________ 31 47 78	Pop:1, Loci: 542 and 117 542 1 2 117 __________ 1.1 29 18 47 2.2 21 15 36 __________ 50 33 83
Pop:1, Loci: 562 and 117 562 1 2 117 __________ 1.1 15 21 36 2.2 11 32 43 __________ 26 53 79	Pop:1, Loci: 562 and 117 562 1 2 117 __________ 1.1 24 27 51 2.2 21 17 38 __________ 45 44 89
Pop:1, Loci: 591 and 117 591 1 2 117 __________ 1.1 26 10 36 2.2 40 3 43 __________ 66 13 79	Pop:1, Loci: 591 and 117 591 1 2 117 __________ 1.1 27 23 50 2.2 16 22 38 __________ 43 45 88
Pop:1, Loci: 338 and 122 338 1 2 122 __________ 1.1 16 22 38 2.2 18 23 41 __________ 34 45 79	Pop:1, Loci: 338 and 122 338 1 2 122 __________ 1.1 26 11 37 2.2 28 14 42 __________ 54 25 79
Pop:1, Loci: 122 and 350 122 1 2 350 __________ 1.1 3 11 14 2.2 35 30 65 __________ 38 41 79	Pop:1, Loci: 122 and 350 122 1 2 350 __________ 1.1 25 26 51 2.2 12 16 28 __________ 37 42 79

Pop:1, Loci: 122 and 513	Pop:1, Loci: 122 and 513
122 1 2 513 __________ 1.1 9 11 20 2.2 29 30 59 __________ 38 41 79	122 1 2 513 __________ 1.1 21 26 47 2.2 16 16 32 __________ 37 42 79
Pop:1, Loci: 130 and 313 130 1 2 313 __________ 1.1 13 14 27 2.2 27 25 52 __________ 40 39 79	Pop:1, Loci: 130 and 313 130 1 2 313 __________ 1.1 34 20 54 2.2 19 15 34 __________ 53 35 88

appendix 8

Phenotypic effects of significant QTL for cycle time in the 2nd cycle depending on sample size. map: map location [cM], LOD: log likelihood ratio statistic.

sample size	significance threshold (permutation test)	trait	linkage group	map	LOD	mean N. vitripennis allele	mean N. longicornis alllele	explained phenotypic variance [%]	associated locus or interval
270	2.4	cycle_2	1	42.3	3.6	9.147	11.591	5.9	CG183
270	2.4	cycle_2	1	24.2	2.1	9.509	11.619	4.5	CE560 EB478
270	2.4	cycle_2	1	47.8	3.3	9.177	11.525	5.4	CE304
270	2.4	cycle_2	2	19.3	2.5	9.694	11.595	4.1	FA472
270	2.4	cycle_2	unmapped	unmapped	8.5	8.838	12.814	14.5	CG348
270	2.4	cycle_2	unmapped	unmapped	6.3	8.983	12.239	10.0	EC196
270	2.4	cycle_2	unmapped	unmapped	3.5	9.049	11.287	5.6	EC216
270	2.4	cycle_2	unmapped	unmapped	3.1	10.964	8.774	6.0	CE584
270	2.4	cycle_2	unmapped	unmapped	3.0	9.343	11.927	4.9	EC236
270	2.4	cycle_2	unmapped	unmapped	2.8	8.943	10.874	4.6	EB285
239	2.4	cycle_2	1	52.8	3.3	9.007	11.658	7.1	CE380
239	2.4	cycle_2	1	24.2	2.1	8.987	11.157	5.4	CE560 EB478
239	2.4	cycle_2	1	47.8	2.9	9.078	11.371	5.4	CE304
239	2.4	cycle_2	2	19.3	1.6	9.166	10.813	3.1	FA472
239	2.4	cycle_2	unmapped	unmapped	6.8	8.826	12.778	14.0	CG348
239	2.4	cycle_2	unmapped	unmapped	5.4	8.954	12.284	10.0	EC196
239	2.4	cycle_2	unmapped	unmapped	2.8	8.971	11.088	5.3	EC216
239	2.4	cycle_2	unmapped	unmapped	2.5	8.865	10.786	4.7	EB285
239	2.4	cycle_2	unmapped	unmapped	2.5	9.280	11.854	4.7	EC236
239	2.4	cycle_2	unmapped	unmapped	2.5	9.228	11.618	4.7	EC288
184	2.3	cycle_2	1	40.3	2.5	9.228	11.810	6.2	FA555
184	2.3	cycle_2	1	47.8	1.2	9.457	11.251	3.0	CE304
184	2.3	cycle_2	1	24.2	2.4	9.065	11.901	7.9	CE560 EB478
184	2.3	cycle_2	2	19.3	2.3	9.104	11.411	5.7	FA472
184	2.3	cycle_2	unmapped	unmapped	6.1	9.002	13.371	14.9	CG348
184	2.3	cycle_2	unmapped	unmapped	4.0	9.180	12.656	9.6	EC196
184	2.3	cycle_2	unmapped	unmapped	3.2	8.933	11.667	8.0	FA340
184	2.3	cycle_2	unmapped	unmapped	3.1	9.049	11.756	7.5	EC216
138	2.4	cycle_2	1	42.3	2.5	8.809	11.297	8.2	CG183
138	2.4	cycle_2	1	62.6	3.1	8.494	11.839	15.2	FA403 CG153
138	2.4	cycle_2	1	26.0	2.3	8.848	11.269	7.6	EB478
138	2.4	cycle_2	1	57.0	2.9	9.032	12.408	9.4	EB272
138	2.4	cycle_2	2	19.3	1.0	9.026	10.592	3.4	FA472
138	2.4	cycle_2	unmapped	unmapped	4.9	8.772	12.844	16.2	CG348

sample size	significance threshold (permutation test)	trait	linkage group	map	LOD	mean N. vitripennis allele	mean N. longicornis alllele	explained phenotypic variance [%]	associated locus or interval
138	2.4	cycle_2	unmapped	unmapped	2.4	10.856	8.453	9.0	CE584
89	2.4	cycle_2	1	40.3	3.0	8.761	12.625	15.4	FA555
89	2.4	cycle_2	?	74.6	3.3	8.283	12.651	24.4	CE411 CE328
89	2.4	cycle_2	1	26.0	2.2	8.748	11.658	10.6	EB478
89	2.4	cycle_2	1	47.8	2.5	8.689	11.808	12.2	CE304
89	2.4	cycle_2	2	36.3	3.1	8.264	12.011	19.5	FA376 CE249

appendix 9

Significant unmapped QTL for courtship components based on a sample size of 298 LV hybrids and the AFLP map (p. 62). LOD: log likelihood ratio statistic.

trait	LOD	allelic effect *N. vitripennis*	allelic effect *N. longicornis*	explained phenotypic variance [%]	locus	reversed allelic effect
cycle_1	6.94	8.0	12.8	12.2	CG348	
cycle_1	4.24	8.4	11.9	6.7	EC196	
cycle_1	3.46	8.5	11.7	5.5	EC199	
cycle_1	3.1	8.7	12.5	5.6	FA164	
cycle_1	2.89	8.5	12.2	6	CG225	
cycle_1	2.89	8.9	13.4	4.6	EC171	
cycle_1	2.89	8.2	10.9	5	FA340	
cycle_1	2.82	8.4	11.3	4.9	FA213	
cycle_2	8.48	8.8	12.8	14.5	CG348	
cycle_2	6.3	9.0	12.2	10	EC196	
cycle_2	3.45	9.0	11.3	5.6	EC216	
cycle_2	3.09	11.0	8.8	6	CE584	
cycle_2	3	9.3	11.9	4.9	EC236	
cycle_2	2.84	8.9	10.9	4.6	EB285	
cycle_3	5.64	8.1	10.6	11.9	EC218	
cycle_3	4.22	8.6	10.8	10.1	FA340	
cycle_3	3.49	8.8	10.9	8.4	FA213	
cycle_3	3.4	9.0	11.5	7.7	CG348	
cycle_3	3.09	8.8	10.8	7.5	FA258	
cycle_3	3.05	8.9	10.9	6.8	CG395	
cycle_3	2.94	8.9	10.9	6.4	EC216	
cycle_4	6.75	8.8	11.5	16.2	FA340	
cycle_4	4.51	9.2	11.6	10.8	CG395	
cycle_4	4.33	9.1	11.4	10.7	FA258	
cycle_4	3.78	9.4	11.9	9.3	CG348	
cycle_4	3.47	8.9	10.7	7.9	EC218	
cycle_4	3.3	9.2	11.2	8.2	FA213	
cycle_4	2.49	9.2	10.8	5.7	EB285	
fix_nod	4.52	4.5	9.7	9.9	CG348	
fix_nod	2.71	4.9	8.7	5	EC236	
fix_nod	2.43	4.7	8.9	6.1	CG225	
h2-h1	2.44	0.5	0.1	4	CG255	

h2-h1	2.36	0.4	-0.1	3.9	CG225	
hnd_1	2.19	2.9	2.4	3.8	CG506	
hnd_2	3.4	2.7	3.4	5.5	CG395	X
hnd_2	2.41	3.1	2.5	4	CE314	
hnd_2	2.3	3.0	2.4	3.7	CG225	
hnd_2	2.27	3.2	2.7	3.5	EB500	
hnd_3	4.79	2.8	3.7	7.9	CG395	X
hnd_3	3.67	2.9	3.7	6.1	CG348	X
hnd_3	3.4	2.8	3.5	5.5	FA340	X
hnd_3	2.83	2.8	3.5	4.8	FA610	X
hnd_3	2.8	2.9	3.6	4.3	EC196	X
hnd_3	2.75	3.3	2.5	4.5	CG225	
hnd_3	2.7	2.6	3.3	4.1	EB118	X
hnd_3	2.66	3.4	2.8	5	CG506	
hnd_3	2.56	3.4	2.8	4.5	CE268	
hnd_3	2.54	3.3	2.7	4.3	CE314	
hnd_3	2.41	3.4	2.8	3.7	EB500	
hnd_3	2.37	2.9	3.6	3.7	EC199	X
hnd_3	2.37	3.4	2.8	3.7	EC327	
hnd_3	2.32	2.9	3.5	3.8	FA213	X
hnd_4	4.64	2.9	3.9	8.3	CG348	
hnd_4	4.36	2.9	3.8	7.8	CG395	X
hnd_4	3.55	2.9	3.6	6.5	FA610	X
hnd_4	3.45	3.0	3.8	5.5	EC196	X
hnd_4	3.27	3.5	2.9	5.2	EB500	X
hnd_4	2.99	2.9	3.6	5.1	FA340	
hnd_4	2.69	3.5	2.9	4.3	EC327	X
latency	3.39	173.3	120.1	5.9	EC392	
latency	2.75	122.7	170.7	4.8	EC120	
latency	2.61	171.8	124.9	4.6	EC324	
latency	2.28	121.8	166.7	4.1	EB359	
latency	2.26	172.7	128.4	4	EC327	
total series	3.46	19.9	11.8	8	EC295	X
total series	2.5	15.1	11.1	5.8	EB198	X
total series	2.01	13.7	10.1	5	FA258	X
minus nods	0			-	EB478	

appendix 10

Courtship components of courtship displays of 481 LV hybrid males

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3d cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3d hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
61.1	-	15.9	11.4	12.0	14.5	3	4	4	5	1	10	-	absent
28.3	-	6.5	6.6	6.9	7.9	5	4	5	6	-1	6	absent	absent
32.0	-	21.1	16.9	17.7	14.9	5	5	6	5	0	6	absent	absent
402.7	-	12.7	11.2	13.7	12.8	4	3	4	3	-1	9	present	absent
20.4	-	17.0	16.6	9.1	8.0	2	3	2	2	1	8	absent	-
62.5	-	7.4	8.8	7.5	8.3	2	5	4	4	3	17	absent	absent
81.7	-	23.0	17.4	11.8	16.0	3	4	5	7	1	11	absent	-
38.5	-	8.1	7.5	8.1	7.4	3	3	3	2	0	24	-	absent

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3^{d} cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3^{d} hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
61.9	-	9.2	9.3	9.6	9.8	3	4	4	4	1	7	absent	present
84.3	-	9.2	8.3	-	7.6	6	2	-	2	-4	10	-	absent
221.9	-	11.9	12.6	12.6	13.2	5	4	4	6	-1	6	present	present
183.9	-	3.1	10.9	11.2	14.0	1	1	2	3	0	8	present	present
389.4	-	10.7	14.0	25.2	-	2	3	1	3	1	4	absent	absent
46.6	-	7.7	7.9	9.1	14.6	2	2	4	4	0	5	absent	present
34.6	-	6.1	5.2	4.8	5.7	1	1	1	1	0	21	absent	-
102.0	-	7.0	7.0	7.4	8.8	2	2	2	2	0	11	absent	absent
89.2	-	6.0	6.9	9.1	9.9	3	3	3	3	0	11	absent	absent
125.7	-	21.2	18.4	21.3	22.2	2	2	3	4	0	22	absent	present
213.7	-	6.7	7.3	8.6	8.3	4	4	5	5	0	15	absent	-
91.8	-	24.8	24.3	-	-	3	4	7	-	1	3	absent	absent
135.3	-	7.1	6.3	7.3	7.7	3	3	4	4	0	6	absent	absent
140.9	-	8.8	10.0	12.1	10.9	4	3	2	3	-1	12	absent	absent
77.1	-	8.1	6.7	6.6	6.9	4	2	2	2	-2	8	absent	-
295.2	-	8.6	10.1	9.8	10.5	3	4	3	3	1	6	absent	present
137.1	0.6	8.4	5.8	5.4	8.2	2	1	2	3	-1	10	absent	present
41.6	0.7	6.4	8.8	10.4	12.2	3	3	3	4	0	19	absent	present
77.2	1.8	6.1	5.8	5.5	7.2	1	2	2	2	1	8	absent	present
212.6	2.8	4.0	4.4	5.6	5.2	1	1	1	1	0	7	absent	present
74.4	-	7.1	8.3	9.1	8.9	2	2	2	2	0	14	absent	absent
274.7	-	5.8	6.2	6.1	6.8	2	2	2	2	0	7	absent	present
66.2	-	1.4	4.7	5.5	6.1	1	1	2	1	0	13	absent	present
104.1	-	4.2	6.6	7.0	8.5	1	3	2	2	2	7	absent	absent
159.3	-	5.6	4.9	5.6	5.9	1	1	1	1	0	14	absent	present
224.4	-	23.6	15.3	16.6	14.4	2	3	4	3	1	11	absent	present
15.5	-	1.7	2.1	4.5	2.4	2	2	2	2	0	9	absent	absent
233.9	-	10.3	12.0	15.4	18.4	3	3	3	3	0	15	absent	absent
276.9	-	6.6	10.2	7.6	6.7	2	2	2	1	0	17	absent	present
229.3	-	5.0	5.7	6.5	5.9	2	2	3	2	0	7	absent	absent
20.3	-	11.0	8.7	8.6	8.3	2	3	3	1	1	23	absent	present
52.7	-	-	-	-	-	3	3	3	4	0	-	absent	-
4.9	-	2.0	1.9	3.4	3.3	2	2	3	2	0	12	absent	-
180.5	-	3.8	4.8	4.3	5.5	1	2	1	2	1	20	absent	-
212.1	1.5	6.1	11.5	12.4	5.8	2	2	3	2	0	21	absent	present
220.7	-	2.9	1.8	1.4	1.9	4	2	1	1	-2	15	absent	present
88.5	0.8	2.8	3.8	4.9	5.0	2	2	2	3	0	40	present	present
68.9	-	6.8	7.1	8.8	8.9	2	4	4	3	2	19	absent	present
193.8	-	3.4	4.6	4.4	4.7	2	2	2	2	0	22	absent	present
51.6	1.8	1.8	1.8	1.2	1.5	2	1	1	1	-1	15	absent	absent
172.9	-	4.1	4.5	5.8	6.3	3	3	3	2	0	13	absent	absent
266.2	-	6.2	5.2	6.2	6.7	2	4	4	4	2	9	absent	-
16.0	-	24.9	2.6	11.8	15.7	1	2	1	1	1	5	present	absent
76.6	-	9.0	6.0	6.6	5.8	2	3	2	2	1	8	absent	present
58.9	-	6.5	5.1	5.6	5.3	3	3	3	3	0	12	absent	absent
193.8	-	14.7	7.0	7.9	8.2	1	2	2	2	1	27	-	-
71.3	3.9	7.0	7.3	5.9	8.0	3	3	2	3	0	7	absent	present
178.1	-	5.8	5.9	5.8	6.8	5	5	4	5	0	14	absent	present

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3d cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3d hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
27.8	-	3.7	2.0	4.6	6.5	2	1	1	1	-1	12	absent	present
299.0	-	17.6	16.0	15.3	14.4	1	6	4	4	5	6	-	present
23.8	-	2.6	6.6	10.3	9.2	3	5	5	6	2	17	absent	absent
57.6	-	10.9	11.7	10.9	11.1	6	6	6	6	0	11	-	absent
21.1	-	14.9	12.9	13.5	13.6	5	6	6	6	1	8	absent	absent
79.6	-	14.1	13.9	14.3	13.3	3	4	5	6	1	5	absent	absent
239.3	9.6	12.1	13.4	12.4	13.7	1	3	2	3	2	10	absent	present
174.0	0.9	6.2	5.5	7.1	7.5	3	3	4	3	0	21	absent	absent
135.6	0.9	5.8	7.3	7.8	7.3	4	4	5	4	0	40	absent	absent
241.4	0.6	4.1	4.6	4.8	4.3	2	4	4	3	2	10	absent	present
108.4	1.5	6.3	8.5	9.0	10.3	3	4	4	5	1	30	absent	absent
118.9	6.6	7.1	7.7	8.2	7.8	2	3	4	3	1	19	absent	absent
114.2	9.3	6.3	6.7	7.6	8.6	2	2	3	3	0	26	absent	absent
280.6	-	-	-	6.7	5.5	2	2	5	2	0	10	absent	present
62.0	11.9	13.0	10.5	11.6	-	2	2	3	2	0	-	absent	present
165.7	2.5	8.2	7.2	7.6	7.7	3	3	3	2	0	11	absent	present
81.5	0.7	8.6	7.0	7.8	8.9	2	2	3	3	0	-	absent	absent
163.6	4.0	2.9	13.4	6.2	11.1	1	2	3	3	1	-	absent	-
62.0	0.3	6.1	5.9	6.7	7.0	5	4	4	4	-1	-	absent	absent
92.6	1.3	5.8	6.8	2.0	6.8	1	2	1	3	1	7	absent	-
99.8	5.6	4.7	8.8	8.1	10.4	2	3	3	4	1	14	absent	-
128.0	7.4	9.4	8.1	9.2	11.9	1	1	1	2	0	-	absent	absent
56.0	0.9	5.7	6.1	7.0	8.3	3	2	3	3	-1	12	absent	absent
145.1	-	8.9	9.5	9.3	-	4	4	4	-	0	-	-	-
166.4	2.4	11.3	11.7	10.5	12.5	4	4	4	5	0	9	present	absent
19.3	0.9	7.1	6.6	7.5	7.0	1	2	2	2	1	5	present	absent
344.7	5.2	13.9	7.3	7.5	7.2	2	2	2	3	0	-	-	-
58.0	1.3	10.4	8.9	10.3	10.7	6	6	7	6	0	-	-	absent
99.3	14.3	18.0	14.5	17.5	12.8	4	3	5	5	-1	7	present	absent
35.6	0.7	6.5	7.6	6.9	7.5	3	4	3	4	1	15	absent	present
232.2	2.9	8.9	10.8	13.3	13.5	2	3	4	3	1	9	present	-
20.3	0.2	-	6.1	4.3	6.7	1	1	2	2	0	5	present	-
190.4	1.9	6.1	9.3	14.7	12.2	1	4	4	5	3	5	present	-
97.5	2.1	4.7	5.6	6.6	7.7	4	4	3	4	0	14	absent	absent
14.4	0.9	6.8	5.1	-	6.0	2	2	3	2	0	12	present	absent
166.5	2.7	9.6	7.3	7.5	10.1	2	2	3	3	0	13	absent	present
93.2	3.1	7.6	7.2	7.6	8.5	2	2	2	2	0	7	absent	absent
43.7	1.7	6.0	7.7	5.7	7.7	2	2	1	3	0	12	absent	present
19.8	5.8	7.6	11.4	-	7.6	5	4	3	4	-1	8	absent	absent
236.1	2.8	7.9	7.5	7.5	8.4	2	2	2	2	0	16	absent	present
47.7	6.4	8.1	7.1	7.0	7.4	2	3	3	4	1	5	-	-
291.2	16.5	8.6	8.7	10.9	-	3	2	3	-	-1	4	-	present
49.6	1.5	5.0	6.4	6.6	7.8	1	3	3	4	2	15	present	absent
17.0	13.8	6.7	6.7	11.6	11.5	1	1	1	1	0	24	present	present
336.0	11.3	6.3	5.9	7.8	8.8	2	1	2	2	-1	8	present	present
12.5	1.2	4.1	6.6	4.8	6.4	2	3	4	3	1	7	present	-
133.2	6.7	8.5	8.8	8.1	-	2	2	2	-	0	4	present	absent
138.8	0.3	6.1	8.3	8.6	10.9	2	3	3	4	1	8	present	absent

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3d cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3d hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
235.5	3.3	2.8	6.1	7.0	7.9	2	2	4	5	0	14	present	absent
344.9	7.8	10.8	10.9	11.9	11.3	5	5	7	7	0	-	present	absent
38.1	2.5	5.9	8.4	10.9	-	3	3	5	-	0	-	present	-
70.3	2.3	7.7	4.9	5.5	6.4	6	4	4	5	-2	-	-	-
32.5	3.7	2.1	13.3	2.3	14.9	1	2	2	1	1	-	present	present
77.7	3.1	11.3	13.7	15.1	14.8	6	4	3	4	-2	9	absent	present
142.9	22.0	55.7	17.0	44.6	20.7	4	3	2	4	-1	5	absent	present
166.1	16.2	10.5	18.7	11.3	13.6	2	3	2	4	1	7	present	present
222.6	5.7	3.5	8.7	13.9	12.3	3	3	5	5	0	15	-	absent
265.8	4.0	11.5	14.5	14.3	13.0	1	3	4	3	2	5	absent	absent
41.0	6.0	6.8	8.7	8.9	8.9	2	4	2	2	2	7	-	absent
229.4	1.1	2.2	6.1	5.6	7.1	1	1	1	2	0	-	-	-
107.8	4.4	6.7	7.3	-	6.7	2	4	3	3	2	18	absent	absent
205.4	1.3	9.0	18.5	22.8	-	6	2	3	-	-4	4	present	absent
71.5	4.0	11.4	13.1	14.4	15.7	4	4	4	3	0	7	present	present
128.8	5.9	9.2	9.5	10.1	10.0	4	4	4	3	0	5	present	absent
16.7	3.8	-	9.2	11.9	7.5	1	2	2	1	1	-	present	absent
85.4	5.8	6.7	9.2	10.5	13.1	2	1	2	2	-1	-	present	-
3.9	15.0	12.6	13.3	-	8.8	5	4	2	2	-1	-	-	present
120.3	2.2	8.2	11.4	14.3	12.0	1	1	3	2	0	-	present	present
60.0	2.3	-	-	7.8	5.5	1	1	2	1	0	-	present	-
273.3	4.8	6.1	8.9	9.3	9.4	3	3	2	2	0	21	absent	-
69.2	15.2	7.6	12.7	-	-	1	1	1	1	0	-	present	present
42.2	1.7	6.3	1.6	5.2	-	1	1	1	1	0	-	present	-
61.6	2.7	10.5	15.0	15.4	17.1	2	6	5	7	4	-	-	absent
95.8	2.4	7.8	9.3	8.4	11.9	1	2	1	2	1	-	-	absent
25.3	21.0	15.3	14.0	14.5	15.0	5	4	5	4	-1	13	absent	present
63.9	3.0	8.3	11.3	12.0	12.1	2	3	3	3	1	15	absent	absent
59.5	1.2	4.6	10.1	6.2	6.6	2	3	3	2	1	30	absent	absent
162.1	4.8	9.3	10.3	11.2	10.9	2	2	2	2	0	22	absent	absent
335.6	1.4	3.3	4.4	1.6	4.4	2	2	2	1	0	11	absent	-
71.6	11.8	13.2	17.2	-	-	3	3	2	3	0	6	present	present
246.2	8.7	13.7	10.8	14.0	11.9	4	5	5	5	1	16	absent	-
254.0	7.0	8.0	10.0	11.1	-	3	4	4	-	1	4	absent	present
36.7	1.4	2.1	3.3	5.9	6.3	2	3	3	4	1	19	absent	present
328.9	6.9	25.5	25.2	38.4	24.5	7	6	5	5	-1	8	-	present
230.7	13.6	19.9	15.3	14.7	13.2	2	4	4	4	2	20	absent	absent
260.8	15.2	7.9	8.5	11.0	10.3	1	3	4	3	2	11	absent	absent
279.4	2.0	-	12.9	-	-	1	2	3	2	1	7	absent	present
316.5	0.8	1.5	9.4	8.1	8.1	1	4	3	3	3	36	-	present
89.9	37.3	-	10.9	9.3	-	1	1	1	1	0	7	present	-
0.1	1.2	4.8	5.5	5.6	6.1	3	2	2	3	-1	38	present	absent
295.4	8.5	12.1	-	10.0	12.3	1	1	3	3	0	13	absent	-
84.7	11.3	13.5	16.9	16.3	18.3	1	1	2	3	0	7	-	-
147.3	7.2	12.0	12.0	13.5	12.3	2	2	3	2	0	10	absent	absent
68.4	1.6	5.6	6.6	5.9	8.2	3	4	3	4	1	12	absent	absent
148.6	0.4	11.2	-	-	19.1	3	3	5	5	0	7	absent	present
141.3	0.8	7.5	8.1	8.8	10.0	3	4	4	5	1	14	present	absent

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3^{d} cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3^{d} hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
162.8	5.4	9.4	10.3	10.1	-	4	5	5	-	1	-	-	absent
74.9	1.9	10.1	6.9	5.2	6.0	1	2	2	3	1	73	absent	-
11.3	2.3	3.5	9.3	9.4	9.6	2	2	3	3	0	8	absent	absent
12.1	2.3	7.4	7.6	8.0	8.2	3	2	3	2	-1	25	absent	absent
3.9	2.8	6.7	7.0	7.9	7.6	2	3	4	4	1	9	present	absent
456.8	7.8	6.4	7.7	8.2	8.1	1	4	5	5	3	32	absent	present
133.4	14.5	-	-	24.3	24.0	2	2	2	4	0	5	absent	absent
80.1	3.4	8.3	9.0	8.1	-	3	3	1	-	0	4	absent	present
210.7	2.3	6.7	9.7	12.1	11.4	1	3	4	4	2	9	absent	absent
173.9	4.4	3.3	7.1	10.6	17.3	2	1	2	2	-1	11	present	-
146.3	1.3	9.6	8.5	11.0	9.2	2	1	1	1	-1	15	absent	absent
87.4	0.2	4.7	5.7	7.9	6.6	3	3	3	2	0	6	absent	absent
66.5	37.6	36.9	34.1	29.8	25.0	6	7	6	5	1	11	absent	absent
244.3	65.9	19.1	15.4	22.8	16.4	7	3	5	6	-4	6	absent	absent
5.8	5.6	6.4	7.7	10.2	11.1	2	2	3	3	0	5	-	absent
277.4	8.2	6.3	9.3	11.5	12.7	2	2	3	4	0	5	-	present
80.1	6.6	8.9	9.8	12.0	12.1	3	1	2	2	-2	35	absent	absent
38.8	10.2	8.8	15.0	16.6	15.8	3	2	4	3	-1	5	-	-
81.5	10.3	9.5	10.6	10.6	11.1	4	5	5	6	1	10	absent	absent
49.6	1.8	6.7	7.0	7.4	8.3	3	3	3	3	0	7	absent	absent
85.0	18.0	14.1	15.0	15.6	16.4	2	3	4	4	1	7	absent	present
0.4	0.3	5.8	6.2	8.1	8.1	4	4	4	4	0	7	absent	absent
242.1	16.4	12.2	14.0	14.5	13.5	3	2	2	2	-1	9	absent	present
45.0	12.0	5.1	7.0	8.2	8.1	2	2	3	2	0	10	absent	absent
295.1	7.9	15.3	17.3	16.3	15.7	1	3	3	3	2	16	absent	present
76.1	11.7	5.3	6.8	7.8	8.4	3	3	2	3	0	14	absent	absent
159.1	3.2	11.1	15.3	15.4	13.5	4	5	5	4	1	19	absent	absent
78.4	7.1	19.0	10.6	14.8	15.9	2	2	2	3	0	8	absent	absent
105.3	2.6	25.8	12.3	12.5	12.9	2	2	2	3	0	22	absent	absent
67.4	1.6	8.8	5.6	6.9	8.4	1	1	1	1	0	14	present	-
115.9	0.7	7.9	8.2	9.0	7.3	1	1	2	1	0	15	present	absent
98.4	1.0	4.7	6.0	6.7	7.2	2	4	2	3	2	16	absent	-
20.7	3.9	5.0	5.3	1.9	5.0	2	2	1	1	0	13	absent	absent
27.8	0.5	-	7.3	9.0	-	2	3	3	2	1	8	absent	absent
51.1	0.7	3.4	5.3	5.8	6.5	3	3	3	4	0	31	absent	absent
33.9	4.9	5.0	6.3	6.0	6.8	2	3	2	3	1	13	absent	absent
56.1	6.2	7.3	8.1	9.5	9.3	1	2	1	1	1	6	-	-
143.0	1.4	7.8	6.7	7.6	9.1	3	2	2	2	-1	14	absent	absent
18.6	1.8	8.6	8.3	7.8	8.4	2	2	2	2	0	9	absent	present
112.7	10.3	5.3	7.8	8.6	8.8	4	4	4	4	0	10	absent	present
213.5	1.0	7.9	8.7	10.8	12.4	3	3	2	1	0	9	absent	present
160.3	4.1	10.8	10.6	11.0	10.4	6	7	7	6	1	18	absent	absent
33.5	6.7	6.2	7.3	8.4	10.0	3	3	3	3	0	8	absent	present
72.7	10.1	9.2	7.7	7.1	10.4	5	2	1	3	-3	9	absent	present
68.5	2.9	5.5	5.5	5.6	5.7	2	2	2	3	0	11	absent	absent
28.0	2.3	3.6	3.9	4.9	5.0	2	3	3	3	1	14	absent	absent
324.2	3.0	8.5	8.4	8.6	9.3	2	2	2	2	0	9	absent	absent
149.0	16.9	16.8	29.8	14.6	24.2	1	1	1	1	0	6	absent	absent

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3d cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3d hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
40.3	28.1	14.4	14.0	13.3	18.0	3	3	2	2	0	16	absent	-
72.8	3.3	11.8	11.0	14.1	11.8	1	1	1	2	0	6	-	-
51.2	1.6	8.7	9.7	9.3	10.7	3	1	2	2	-2	9	absent	present
105.1	0.5	3.6	4.7	6.5	7.2	3	2	3	2	-1	5	absent	present
93.7	2.9	25.5	17.1	14.6	16.9	2	4	4	4	2	45	present	absent
44.9	6.7	7.9	8.3	9.0	8.7	3	3	4	2	0	11	absent	absent
173.1	4.3	7.1	2.0	-	-	1	1	1	1	0	10	present	present
37.6	3.9	8.3	7.1	7.8	8.1	2	2	2	2	0	10	absent	-
202.4	5.0	8.4	8.3	7.7	8.5	2	2	1	2	0	20	present	present
90.3	1.6	3.4	4.9	4.1	5.9	1	2	1	2	1	12	present	-
15.3	0.8	5.6	6.2	7.4	8.4	3	2	3	3	-1	12	absent	absent
128.8	0.4	7.3	12.6	11.4	12.5	4	9	7	8	5	11	-	absent
301.7	4.0	16.7	21.1	21.0	12.7	2	2	3	2	0	12	-	present
271.3	4.4	10.4	9.7	14.4	11.2	5	4	4	4	-1	11	absent	-
15.2	2.5	8.7	8.6	14.2	8.8	1	1	1	1	0	12	absent	absent
78.9	2.8	7.5	9.5	11.8	25.2	4	4	5	5	0	8	absent	absent
275.9	4.2	6.1	8.0	9.8	10.8	1	2	2	2	1	15	absent	present
105.0	4.2	12.0	25.4	16.6	17.8	4	7	5	5	3	6	present	present
275.3	0.6	12.1	13.9	13.2	14.7	4	2	3	2	-2	11	absent	present
25.5	0.2	5.7	6.4	7.1	7.5	2	2	2	2	0	12	absent	absent
77.4	0.2	8.5	8.8	9.9	12.6	1	1	1	1	0	12	absent	absent
267.0	8.5	-	9.6	13.9	-	1	2	3	4	1	4	present	absent
67.2	8.7	5.5	12.8	5.0	6.1	3	3	3	4	0	7	present	absent
0.3	0.3	6.3	6.1	6.9	8.2	3	2	3	2	-1	10	absent	absent
160.0	4.8	6.3	16.0	6.5	-	1	2	2	2	1	4	present	absent
51.1	9.8	5.6	7.8	9.3	13.9	3	5	5	6	2	8	absent	absent
84.0	5.0	11.2	12.4	12.6	9.9	2	2	2	3	0	17	absent	-
42.4	7.0	12.6	11.6	10.4	11.5	5	3	4	4	-2	10	absent	present
84.4	8.4	23.2	27.3	18.4	11.9	1	3	3	1	2	5	absent	absent
14.4	2.4	8.3	5.2	6.6	7.4	7	3	3	3	-4	8	absent	absent
93.1	3.3	8.5	9.4	9.4	9.4	1	2	2	2	1	9	absent	-
265.3	3.6	8.4	10.7	13.1	9.1	1	2	2	1	1	5	absent	absent
126.4	1.6	6.7	7.6	8.4	8.8	2	1	3	3	-1	7	absent	-
265.8	2.8	-	13.2	15.2	14.5	9	6	7	6	-3	10	absent	present
30.2	3.5	9.3	8.2	7.8	13.3	3	2	3	2	-1	10	-	present
24.0	0.2	3.6	4.3	4.8	5.5	2	3	2	2	1	7	absent	absent
365.2	7.4	8.0	9.1	10.0	10.5	2	2	2	2	0	22	absent	absent
54.5	8.5	9.9	9.7	10.7	10.4	3	4	4	4	1	18	absent	absent
56.9	4.6	10.0	7.0	7.3	8.3	1	3	2	3	2	25	absent	-
94.7	5.5	2.6	4.7	5.2	6.3	2	3	3	3	1	12	present	absent
229.5	2.0	5.4	5.4	6.4	11.5	3	3	3	4	0	-	absent	absent
69.4	11.6	11.1	11.0	12.0	11.9	5	5	3	4	0	-	absent	absent
9.6	1.4	6.5	7.2	7.1	8.8	3	3	4	4	0	15	absent	absent
161.9	1.7	7.1	9.7	9.4	10.3	4	6	5	6	2	6	absent	absent
87.7	10.9	37.6	39.6	-	-	2	3	3	-	1	3	present	present
59.6	1.6	6.2	8.9	9.3	9.1	3	4	4	4	1	10	-	absent
131.6	8.3	9.2	-	9.4	9.3	3	-	3	2	-	16	absent	absent
136.9	1.5	5.9	7.3	7.9	9.0	1	1	1	2	0	-	-	absent

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3d cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3d hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
193.2	2.5	9.2	13.1	15.0	14.8	4	4	5	4	0	6	present	present
37.7	2.2	10.2	9.5	8.7	9.1	3	3	3	3	0	8	-	present
73.7	9.1	-	7.7	8.5	9.5	2	2	2	2	0	14	-	absent
185.0	0.7	9.8	10.0	9.6	9.1	4	4	2	2	0	29	-	-
145.3	1.1	2.9	5.7	6.4	6.7	3	2	3	3	-1	54	absent	absent
23.2	6.9	12.2	11.5	10.5	10.1	4	5	5	5	1	10	absent	absent
101.9	3.1	9.6	9.0	11.3	10.6	2	2	2	2	0	16	absent	absent
90.1	2.9	7.8	8.3	8.7	8.7	1	1	1	1	0	15	present	present
30.6	9.6	5.8	8.0	7.7	8.2	3	3	5	4	0	10	absent	absent
300.3	1.3	3.1	2.3	3.2	5.1	1	1	2	3	0	45	absent	absent
138.7	6.8	6.6	8.3	9.1	10.4	2	2	1	2	0	23	absent	absent
0.5	0.3	5.3	5.1	4.9	9.7	3	3	2	3	0	31	present	absent
264.6	7.3	11.0	15.9	11.7	12.6	1	2	2	2	1	12	absent	present
182.4	7.5	8.1	13.2	14.4	15.2	1	7	7	6	6	5	absent	-
80.0	3.9	12.9	21.3	10.0	9.1	6	5	4	3	-1	18	-	present
19.3	1.4	5.5	6.2	6.9	7.2	2	2	2	2	0	18	absent	absent
229.0	41.7	79.4	67.7	30.0	-	2	1	3	3	-1	4	-	absent
77.0	2.8	8.5	9.6	10.4	9.7	2	3	3	3	1	7	absent	absent
248.9	4.5	8.9	11.5	11.7	13.5	3	4	5	5	1	13	absent	absent
68.5	2.8	7.2	11.0	11.5	12.2	2	3	3	3	1	12	absent	present
305.9	3.6	8.1	8.8	14.1	13.4	3	2	4	4	-1	9	absent	absent
33.1	0.1	6.5	5.0	7.3	6.5	3	2	4	4	-1	16	absent	absent
77.8	3.1	5.6	6.2	6.4	7.0	2	3	3	3	1	12	absent	absent
19.9	2.9	6.3	9.6	9.9	9.5	2	4	5	4	2	12	present	absent
192.5	12.0	8.5	8.4	10.1	9.0	3	3	3	2	0	17	absent	absent
195.3	2.4	7.5	9.0	9.2	10.8	5	5	5	4	0	5	absent	absent
266.4	0.2	-	9.4	11.1	11.8	3	7	6	6	4	22	absent	absent
44.9	7.7	9.5	2.7	16.1	12.2	1	1	1	1	0	26	-	absent
24.8	1.6	5.5	3.3	5.6	5.6	2	2	2	2	0	8	absent	absent
46.4	2.9	10.8	10.3	10.6	11.8	5	4	4	4	-1	9	absent	absent
129.8	4.1	9.6	1.9	12.3	14.4	1	1	1	2	0	9	-	absent
211.3	2.0	6.6	8.3	9.2	9.5	2	2	2	2	0	6	-	-
25.0	2.0	11.1	10.4	10.5	11.8	2	2	4	4	0	11	absent	absent
44.0	0.1	2.2	5.2	5.5	6.0	2	3	2	2	1	12	absent	absent
209.9	0.8	4.9	5.7	7.7	7.5	3	3	4	5	0	17	absent	present
151.8	0.3	7.1	7.2	7.0	7.8	4	3	1	3	-1	12	absent	absent
257.8	34.5	13.2	19.2	13.3	11.2	2	3	2	1	1	18	present	present
88.9	3.0	1.5	6.3	9.5	10.0	1	1	3	5	0	9	present	absent
232.1	21.2	11.8	9.2	7.7	8.8	1	1	4	4	0	17	absent	absent
232.3	4.7	-	12.0	12.7	12.8	1	1	1	2	0	18	absent	absent
206.4	17.8	18.5	16.9	13.5	-	5	3	3	-	-2	4	absent	absent
257.8	1.8	7.0	6.5	7.7	8.6	2	1	2	2	-1	8	-	absent
61.3	2.0	6.2	6.9	7.6	9.1	3	3	2	2	0	6	absent	absent
116.2	1.3	6.4	7.1	7.4	7.6	6	6	6	6	0	9	absent	absent
64.8	1.7	11.9	11.6	13.9	12.5	4	4	4	4	0	11	-	absent
94.7	2.0	18.2	15.3	14.1	17.4	8	6	6	4	-2	27	absent	absent
74.9	3.5	13.3	2.1	8.2	8.2	1	1	1	2	0	12	absent	absent
22.3	0.9	3.8	4.5	4.8	5.5	2	2	2	3	0	12	absent	absent

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3^{d} cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3^{d} hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
239.0	2.7	6.2	6.2	7.1	7.3	2	2	2	1	0	7	absent	absent
154.3	0.8	6.2	7.8	7.9	9.8	2	3	2	4	1	10	absent	absent
181.0	1.6	1.8	5.5	4.6	4.5	1	1	1	1	0	9	absent	absent
120.6	3.5	17.9	20.0	21.3	18.4	3	3	5	2	0	9	absent	absent
30.5	0.7	7.6	8.3	8.8	8.8	2	3	4	2	1	28	absent	absent
150.1	21.7	5.1	6.0	5.8	5.8	4	4	3	3	0	11	absent	absent
243.4	4.7	9.0	28.4	16.0	-	3	3	2	-	0	4	absent	present
160.7	9.9	14.6	13.5	15.3	14.9	5	6	7	4	1	10	absent	absent
69.7	4.9	16.2	11.4	11.5	10.1	1	3	2	2	2	15	absent	absent
245.5	4.9	8.6	11.0	11.4	13.7	2	3	2	3	1	14	-	present
23.2	0.4	5.9	7.2	7.1	7.8	2	3	3	3	1	12	absent	absent
180.4	0.9	6.7	7.0	7.9	8.3	4	6	4	4	2	21	absent	absent
148.6	1.7	8.9	10.3	9.5	9.9	2	3	3	3	1	47	absent	absent
142.4	2.7	5.8	6.3	3.8	-	2	1	2	-	-1	18	absent	absent
75.0	8.0	9.0	9.0	-	-	2	6	5	6	4	-	absent	absent
120.0	5.0	13.0	13.0	-	-	2	2	2	3	0	-	present	absent
75.0	5.0	7.0	8.0	-	-	3	3	3	3	0	-	present	absent
180.0	5.0	5.0	5.0	-	-	2	2	3	3	0	-	absent	absent
180.0	5.0	6.0	7.0	-	-	1	2	1	-	1	-	present	absent
-	-	9.0	12.0	-	-	2	2	2	-	0	-	present	present
-	6.0	11.0	12.0	-	-	3	2	3	3	-1	-	absent	absent
-	-	7.0	7.0	-	-	2	4	4	4	2	-	absent	absent
-	-	5.0	5.0	-	-	4	4	3	-	0	-	absent	-
300.0	6.0	9.0	11.0	-	-	1	2	3	3	1	-	present	present
-	6.0	6.0	6.0	-	-	3	3	1	4	0	-	absent	absent
300.0	3.0	4.0	5.0	-	-	1	1	3	3	0	-	absent	present
540.0	12.0	25.0	-	-	-	3	4	2	3	1	-	absent	present
120.0	5.0	4.0	6.0	-	-	3	3	3	3	0	-	absent	absent
240.0	10.0	11.0	12.0	-	-	1	2	1	1	1	-	absent	present
240.0	12.0	7.0	8.0	-	-	1	2	2	2	1	-	absent	present
150.0	5.0	11.0	10.0	-	-	2	2	2	3	0	-	absent	absent
190.0	15.0	-	15.0	-	-	1	2	2	2	1	-	absent	absent
250.0	4.0	16.0	14.0	-	-	2	2	1	-	0	-	present	absent
240.0	11.0	13.0	15.0	-	-	1	2	3	3	1	-	absent	absent
90.0	4.0	5.0	5.0	-	-	1	2	3	3	1	-	absent	absent
-	15.0	10.0	11.0	-	-	3	2	1	-	-1	-	absent	absent
360.0	10.0	10.0	11.0	-	-	2	1	2	1	-1	-	absent	present
540.0	7.0	15.0	15.0	-	-	2	3	3	3	1	-	absent	present
150.0	4.0	13.0	14.0	-	-	3	2	3	4	-1	-	absent	present
390.0	7.0	8.0	10.0	-	-	1	1	2	2	0	-	present	absent
570.0	-	9.0	9.0	-	-	3	3	3	4	0	-	present	absent
540.0	5.0	6.0	8.0	-	-	2	4	3	3	2	-	present	present
-	4.0	15.0	15.0	-	-	2	3	4	4	1	-	absent	absent
480.0	12.0	16.0	14.0	-	-	4	4	5	3	0	-	present	absent
600.0	13.0	17.0	18.0	-	-	4	4	4	5	0	-	present	present
240.0	15.0	6.0	6.0	-	-	2	2	2	2	0	-	present	present
1200.0	6.0	9.0	10.0	-	-	1	1	1	2	0	-	present	present
130.0	6.0	6.5	7.0	-	-	2	3	2	-	1	-	absent	absent

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3^{d} cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3^{d} hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
165.0	3.0	8.0	15.5	-	-	3	2	3	2	-1	-	absent	absent
-	3.0	5.0	6.0	-	-	3	3	3	3	0	-	absent	absent
-	-	9.0	16.0	-	-	2	3	2	2	1	-	absent	absent
120.0	25.0	12.0	14.5	-	-	4	4	4	5	0	-	present	present
270.0	7.0	7.0	8.5	-	-	3	3	3	3	0	-	absent	present
45.0	6.0	13.0	13.0	-	-	4	6	1	-	2	-	absent	present
300.0	4.0	8.0	9.0	-	-	2	2	1	2	0	-	absent	present
-	-	10.0	12.0	-	-	2	3	2	-	1	-	absent	absent
-	-	21.0	22.0	-	-	6	5	6	6	-1	-	absent	absent
300.0	-	15.0	16.0	-	-	-	1	1	2	-	-	present	absent
-	-	13.0	-	-	-	4	3	3	5	-1	-	present	absent
120.0	-	12.0	-	-	-	4	4	-	4	0	-	absent	absent
120.0	-	9.0	9.0	-	-	4	4	4	5	0	-	absent	absent
-	4.0	7.0	8.0	-	-	3	3	3	4	0	-	absent	absent
120.0	-	6.0	8.0	-	-	4	4	4	4	0	-	absent	absent
-	10.0	8.0	9.0	-	-	3	3	2	3	0	-	absent	absent
480.0	8.0	-	11.0	-	-	2	2	2	1	0	-	absent	absent
480.0	15.0	25.0	30.0	-	-	8	10	11	11	2	-	absent	absent
-	10.0	30.0	38.0	-	-	6	3	3	4	-3	-	present	present
-	6.0	4.5	5.0	-	-	1	1	1	1	0	-	present	present
240.0	7.0	10.0	-	-	-	8	4	4	3	-4	-	-	present
-	8.0	22.0	25.0	-	-	4	4	2	3	0	-	absent	present
-	-	-	-	-	-	2	3	3	3	1	-	absent	present
120.0	-	23.0	21.0	-	-	3	2	2	2	-1	-	absent	present
150.0	10.0	37.0	-	-	-	2	2	3	3	0	-	absent	present
480.0	30.0	129.0	55.0	-	-	5	3	5	5	-2	-	present	present
-	70.0	78.0	-	-	-	3	4	3	-	1	-	present	present
240.0	27.0	28.0	40.0	-	-	1	2	3	3	1	-	absent	present
180.0	5.0	9.0	11.0	-	-	3	3	3	3	0	-	present	absent
120.0	25.0	15.0	17.0	-	-	2	2	3	3	0	-	present	present
-	5.0	9.5	11.0	-	-	1	2	3	3	1	-	present	present
-	18.0	15.0	15.5	-	-	7	4	3	5	-3	-	absent	present
-	-	15.0	15.0	-	-	2	2	3	3	0	-	present	present
360.0	17.0	20.0	20.0	-	-	3	4	4	4	1	-	present	present
600.0	4.0	6.5	7.5	-	-	1	1	1	1	0	-	present	present
120.0	-	27.0	31.0	-	-	2	3	4	4	1	-	present	present
240.0	-	17.0	17.0	-	-	5	5	6	5	0	-	present	absent
-	15.0	26.0	26.0	-	-	1	1	1	1	0	-	present	absent
-	5.0	9.0	12.0	-	-	5	5	6	8	0	-	present	present
-	15.0	15.0	-	-	-	6	8	12	10	2	-	present	absent
120.0	3.0	6.0	7.0	-	-	5	5	3	3	0	-	present	absent
-	3.0	6.0	7.0	-	-	2	3	2	2	1	-	present	present
300.0	4.0	9.0	10.0	-	-	1	2	4	4	1	-	present	absent
-	30.0	11.0	11.5	-	-	1	2	3	3	1	-	absent	absent
-	24.0	18.0	30.0	-	-	4	5	3	4	1	-	present	absent
-	5.0	7.0	8.0	-	-	3	3	3	3	0	-	absent	absent
240.0	5.0	16.0	31.0	-	-	2	4	3	7	2	-	absent	present
-	3.0	9.0	9.0	-	-	3	1	1	3	-2	-	absent	present

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3^{d} cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3^{d} hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
-	3.0	12.0	14.0	-	-	6	6	9	8	0	-	present	absent
300.0	10.0	10.0	10.0	-	-	3	4	3	4	1	-	present	absent
150.0	3.0	7.5	9.0	-	-	3	4	4	4	1	-	present	absent
360.0	-	7.0	9.0	-	-	2	2	3	3	0	-	absent	absent
300.0	4.0	9.0	10.0	-	-	2	4	2	-	2	-	present	present
240.0	-	6.0	7.0	-	-	2	2	3	2	0	-	present	present
-	3.0	4.0	5.0	-	-	3	2	2	2	-1	-	absent	absent
240.0	5.0	11.0	-	-	-	3	3	5	4	0	-	present	present
420.0	6.0	11.0	11.0	-	-	2	1	-	-	-1	-	absent	absent
-	2.0	8.0	9.0	-	-	4	4	4	4	0	-	absent	absent
-	-	19.0	19.0	-	-	9	10	8	9	1	-	absent	absent
240.0	5.0	11.0	17.0	-	-	7	3	2	2	-4	-	present	absent
-	14.0	11.0	13.0	-	-	2	2	3	3	0	-	present	-
-	4.0	14.0	12.0	-	-	4	4	3	3	0	-	absent	absent
60.0	-	8.5	10.0	-	-	3	4	5	4	1	-	absent	absent
-	5.0	15.0	15.0	-	-	10	10	7	8	0	-	absent	absent
240.0	5.0	16.0	16.5	-	-	5	4	4	5	-1	-	present	present
180.0	4.0	10.0	13.0	-	-	3	4	2	-	1	-	present	absent
-	5.0	11.0	15.0	-	-	2	4	5	6	2	-	absent	-
-	8.0	12.0	14.5	-	-	2	2	2	2	0	-	present	absent
-	4.0	12.0	14.0	-	-	3	5	5	5	2	-	present	absent
-	5.0	6.0	8.0	-	-	2	3	4	4	1	-	present	absent
-	3.0	8.0	10.0	-	-	4	3	3	4	-1	-	absent	-
-	6.0	7.0	8.0	-	-	2	2	3	3	0	-	present	-
180.0	-	4.0	4.0	-	-	1	1	1	1	0	-	present	absent
-	11.0	9.5	10.0	-	-	2	1	2	1	-1	-	absent	present
-	3.0	7.0	7.5	-	-	4	2	-	3	-2	-	absent	present
180.0	14.0	17.0	25.0	-	-	1	3	3	3	2	-	present	present
-	10.0	13.0	14.0	-	-	1	1	1	2	0	-	present	present
120.0	5.0	7.0	9.0	-	-	3	4	4	5	1	-	absent	present
300.0	5.0	8.0	8.5	-	-	1	1	1	1	0	-	absent	present
240.0	-	8.0	10.0	-	-	2	2	3	3	0	-	absent	present
300.0	1.0	4.5	6.0	-	-	3	3	4	4	0	-	absent	absent
-	2.0	6.0	7.5	-	-	3	4	4	5	1	-	absent	absent
-	4.0	8.0	10.0	-	-	1	1	2	2	0	-	absent	-
-	3.0	6.0	10.0	-	-	1	1	1	1	0	-	absent	present
120.0	10.0	25.0	-	-	-	6	5	5	5	-1	-	present	present
180.0	-	10.0	12.0	-	-	2	3	3	3	1	-	present	absent
-	12.0	14.0	15.0	-	-	4	6	6	-	2	-	absent	present
-	4.0	7.0	7.0	-	-	1	1	2	2	0	-	present	present
300.0	3.0	7.0	9.0	-	-	1	2	3	5	1	-	absent	present
-	2.0	4.0	4.5	-	-	3	3	3	3	0	-	absent	absent
60.0	3.0	10.0	13.0	-	-	3	3	4	5	0	-	absent	present
-	-	13.0	-	-	-	5	5	-	-	0	-	absent	present
-	-		15.0	-	-	2	2	3	2	0	-	present	absent
180.0	3.0	10.0	10.5	-	-	3	4	3	4	1	-	present	absent
-	2.0	6.0	6.5	-	-	2	2	2	2	0	-	present	present
-	2.0	8.0	8.5	-	-	3	7	4	5	4	-	present	present

latency [sec]	fix 1st nod [sec]	1st cycle [sec]	2nd cycle [sec]	3d cycle [sec]	4th cycle [sec]	1st hnd	2nd hnd	3d hnd	4th hnd	h2-h1	total series	forefeet rubbing	minus nods
-	3.0	5.0	6.5	-	-	3	3	4	4	0	-	absent	absent
480.0	3.0	9.0	10.0	-	-	3	2	3	3	-1	-	absent	present
300.0	5.0	7.5	12.0	-	-	2	2	2	3	0	-	present	absent
180.0	7.0	13.0	17.0	-	-	3	5	7	8	2	-	present	present
60.0	7.0	7.0	7.0	-	-	-	2	4	4	-	-	present	absent
120.0	8.0	10.5	-	-	-	2	2	1	1	0	-	-	-
240.0	8.0	9.5	-	-	-	1	3	2	-	2	-	present	absent
120.0	5.0	5.0	6.0	-	-	2	3	3	4	1	-	-	present
180.0	13.0	17.0	17.5	-	-	3	4	6	1	1	-	present	present
120.0	3.0	5.0	6.0	-	-	1	2	2	2	1	-	present	absent
120.0	4.0	7.5	8.0	-	-	2	2	3	3	0	-	present	absent
180.0	3.0	11.5	12.0	-	-	1	3	3	3	2	-	present	present
-	2.0	6.0	6.5	-	-	4	2	4	4	-2	-	absent	present
120.0	-	6.0	8.0	-	-	4	4	4	4	0	-	absent	present
360.0	1.0	10.0	11.0	-	-	3	3	3	3	0	-	present	present
120.0	3.0	6.0	6.5	-	-	1	1	1	1	0	-	present	absent
120.0	4.0	6.5	7.5	-	-	2	3	2	4	1	-	absent	absent
-	4.0	8.0	10.0	-	-	3	4	4	3	1	-	present	absent
240.0	4.9	5.0	10.0	-	-	3	4	4	4	1	-	present	absent
120.0	1.0	5.0	5.5	-	-	3	2	3	2	-1	-	absent	present
720.0	2.0	7.0	7.5	-	-	3	3	3	3	0	-	absent	absent
60.0	2.0	6.0	6.5	-	-	3	4	3	4	1	-	present	present
480.0	2.0	7.0	8.0	-	-	3	5	4	4	2	-	absent	absent
120.0	2.0	10.0	11.5	-	-	6	7	5	6	1	-	absent	absent
-	5.0	6.5	7.0	-	-	1	1	1	1	0	-	present	present
240.0	2.0	14.0	18.0	-	-	4	6	6	5	2	-	present	present
-	2.0	7.0	7.5	-	-	5	3	4	4	-2	-	absent	present
120.0	4.0	11.5	12.0	-	-	6	5	4	3	-1	-	absent	present
-	-	6.0	7.5	-	-	3	3	3	4	0	-	-	present
-	-	12.0	13.0	-	-	3	3	4	3	0	-	present	present
240.0	3.0	6.5	8.5	-	-	3	3	2	2	0	-	present	absent
180.0	-	8.0	9.0	-	-	4	4	5	4	0	-	absent	absent
-	-	7.0	8.0	-	-	5	5	6	6	0	-	absent	absent
-	3.0	6.5	7.5	-	-	2	3	3	4	1	-	present	absent
585.0	3.0	10.5	11.0	-	-	4	4	5	5	0	-	present	present
-	3.0	8.0	8.0	-	-	2	4	4	4	2	-	present	present
-	3.0	11.0	11.5	-	-	4	5	4	5	1	-	present	present
-	6.0	7.5	9.0	-	-	2	5	5	4	3	-	present	absent
-	-	11.5	11.5	-	-	1	1	1	1	0	-	present	present
-	-	-	-	-	-	1	1	1	1	0	-	present	absent

appendix 11

Wing size charachters and courtship components of a subset of 94 LV hybrid males out of 481 individuals phenotyped for courtship behaviour

intercoular distance [mm]	wing length [mm]	wing width [mm]	length x width normalized by interocular dist.ance	seta density	latency [sec]	fix 1st nod [sec]	1st_cycle [sec]	2nd_cycle [length]	1st_hnd	2nd_hnd	3d_hnd	4th_hnd	h2-h1	forefeet rubbing	minus nods
0.36	1.13	0.38	1.20	68	240	11	13.0	15.0	1	2	3	3	1	absent	absent
0.38	0.91	0.28	0.67	109	300	-	15.0	16.0	-	1	1	2	-	present	absent
0.41	0.96	0.33	0.76	137	-	-	13.0	-	4	3	3	5	1	present	absent
0.41	1.11	0.38	1.04	104	120	-	12.0	-	4	4	-	4	0	absent	absent
0.48	1.16	0.34	0.82	97	120	-	9.0	9.0	4	4	4	5	0	absent	absent
0.36	1.11	0.36	1.10	118	-	4	7.0	8.0	3	3	3	4	0	absent	absent
0.43	0.97	0.29	0.65	84	120	-	6.0	8.0	4	4	4	4	0	absent	absent
0.41	1.03	0.28	0.70	99	-	10	8.0	9.0	3	3	2	3	0	absent	absent
0.36	0.99	0.32	0.89	80	480	8	-	11.0	2	2	2	1	0	absent	absent
0.43	1.30	0.51	1.54	94	240	7	10.0	-	8	4	4	3	4	-	present
0.41	0.93	0.31	0.72	106	-	-	-	-	2	3	3	3	1	absent	present
0.41	1.07	0.36	0.94	84	120	-	23.0	21.0	3	2	2	2	1	absent	present
0.38	1.13	0.37	1.10	67	150	10	37.0	-	2	2	3	3	0	absent	present
0.38	-	-	-	-	-	70	78.0	-	3	4	3	-	1	present	present
0.41	0.94	0.28	0.65	109	240	27	28.0	40.0	1	2	3	3	1	absent	present
0.29	1.14	0.36	1.42	61	180	5	9.0	11.0	3	3	3	3	0	present	absent
0.38	1.10	0.41	1.16	74	120	25	15.0	17.0	2	2	3	3	0	present	present
0.31	0.91	0.28	0.83	71	-	18	15.0	15.5	7	4	3	5	3	absent	present
0.41	1.19	0.43	1.25	89	-	-	15.0	15.0	2	2	3	3	0	present	present
0.46	1.21	0.47	1.26	68	360	17	20.0	20.0	3	4	4	4	1	present	present
0.38	-	-	-	102	600	4	6.5	7.5	1	1	1	1	0	present	present
0.34	0.91	0.29	0.78	85	120	-	27.0	31.0	2	3	4	4	1	present	present
0.43	1.10	0.34	0.87	75	-	5	9.0	12.0	5	5	6	8	0	present	present
0.36	1.11	0.39	1.22	89	-	15	15.0	-	6	8	12	10	2	present	absent
0.41	1.05	0.31	0.81	87	120	3	6.0	7.0	5	5	3	3	0	present	absent
0.46	1.00	0.30	0.67	97	-	3	6.0	7.0	2	3	2	2	1	present	present
0.41	1.29	0.50	1.59	62	300	4	9.0	10.0	1	2	4	4	1	present	absent
0.34	0.80	0.21	0.50	111	-	30	11.0	11.5	1	2	3	3	1	absent	absent
0.34	0.98	0.28	0.82	99	-	24	18.0	30.0	4	5	3	4	1	present	absent
0.34	0.89	0.26	0.70	84	-	5	7.0	8.0	3	3	3	3	0	absent	absent
0.34	0.87	0.27	0.70	84	240	5	16.0	31.0	2	4	3	7	2	absent	present
0.46	1.11	0.32	0.77	129	-	3	9.0	9.0	3	1	1	3	2	absent	present
0.36	-	0.35	0.00	85	-	3	12.0	14.0	6	6	9	8	0	present	absent
0.38	1.20	0.35	1.10	128	300	10	10.0	10.0	3	4	3	4	1	present	absent
0.38	1.07	0.17	0.47	93	360	-	7.0	9.0	2	2	3	3	0	absent	absent
0.38	1.16	0.32	0.98	120	300	4	9.0	10.0	2	4	2	-	2	present	present
0.34	0.91	0.26	0.72	94	240	-	6.0	7.0	2	2	3	2	0	present	present
0.34	1.05	0.33	1.03	132	240	5	11.0	-	3	3	5	4	0	present	present
0.43	0.93	0.22	0.48	107	420	6	11.0	11.0	2	1	-	-	1	absent	absent
0.46	1.16	0.32	0.81	91	-	2	8.0	9.0	4	4	4	4	0	absent	absent
0.41	-	0.34	0.00	107	-	4	14.0	12.0	4	4	3	3	0	absent	absent
0.34	1.13	0.32	1.07	-	60	-	8.5	10.0	3	4	5	4	1	absent	absent
0.36	1.09	0.31	0.93	93	-	5	15.0	15.0	10	10	7	8	0	absent	absent

C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16
-	-	-	-	-	240	5	16.0	16.5	5	4	4	5	1	present	present
0.43	0.98	0.24	0.54	112	180	4	10.0	13.0	3	4	2	-	1	present	absent
0.38	1.07	0.38	1.05	105	-	5	11.0	15.0	2	4	5	6	2	absent	-
0.38	1.03	0.34	0.92	90	-	8	12.0	14.5	2	2	2	2	0	present	absent
0.46	1.07	0.34	0.79	47	-	4	12.0	14.0	3	5	5	5	2	present	absent
0.43	0.95	0.31	0.67	117	-	5	6.0	8.0	2	3	4	4	1	present	absent
0.46	1.15	0.31	0.79	109	-	3	8.0	10.0	4	3	3	4	1	absent	-
0.41	1.11	0.33	0.90	103	-	6	7.0	8.0	2	2	3	3	0	present	-
0.41	1.04	0.39	0.98	102	-	11	9.5	10.0	2	1	2	1	1	absent	present
0.46	1.15	0.20	0.51	115	-	3	7.0	7.5	4	2	-	3	2	absent	present
0.36	-	0.39	0.00	84	-	10	13.0	14.0	1	1	1	2	0	present	present
0.43	-	0.39	0.00	113	120	5	7.0	9.0	3	4	4	5	1	absent	present
0.38	1.06	0.34	0.93	89	300	5	8.0	8.5	1	1	1	1	0	absent	present
0.38	1.00	0.33	0.87	98	240	-	8.0	10.0	2	2	3	3	0	absent	present
0.46	1.16	0.40	1.01	97	300	1	4.5	6.0	3	3	4	4	0	absent	absent
0.48	1.20	0.34	0.85	85	-	2	6.0	7.5	3	4	4	5	1	absent	absent
0.34	0.99	0.29	0.84	88	-	4	8.0	10.0	1	1	2	2	0	absent	-
0.38	0.94	0.24	0.58	110	-	3	6.0	10.0	1	1	1	1	0	absent	present
0.38	1.04	0.31	0.84	-	180	-	10.0	12.0	2	3	3	3	1	present	absent
0.43	0.98	0.38	0.86	110	-	12	14.0	15.0	4	6	6	-	2	absent	present
0.43	1.08	0.36	0.89	106	-	4	7.0	7.0	1	1	2	2	0	present	present
0.34	0.95	0.34	0.97	104	300	3	7.0	9.0	1	2	3	5	1	absent	present
0.46	1.31	0.39	1.13	100	-	2	4.0	4.5	3	3	3	3	0	absent	absent
0.34	1.01	0.34	1.03	70	60	3	10.0	13.0	3	3	4	5	0	absent	present
0.36	1.08	0.35	1.05	71	-	-	13.0	-	5	5	-	-	0	absent	present
0.43	1.03	0.38	0.90	79	-	-	-	15.0	2	2	3	2	0	present	absent
0.41	1.05	0.34	0.88	134	180	3	10.0	10.5	3	4	3	4	1	present	absent
0.41	1.18	0.33	0.94	101	-	2	6.0	6.5	2	2	2	2	0	present	present
0.41	1.06	0.33	0.87	91	-	2	8.0	8.5	3	7	4	5	4	present	present
0.48	1.13	0.32	0.76	99	-	3	5.0	6.5	3	3	4	4	0	absent	absent
0.46	1.14	0.37	0.92	105	480	3	9.0	10.0	3	2	3	3	1	absent	present
0.41	-	-	-	-	300	5	7.5	12.0	2	2	2	3	0	present	absent
0.38	1.01	0.38	1.01	89	180	7	13.0	17.0	3	5	7	8	2	present	present
0.41	1.08	0.32	0.84	112	60	7	7.0	7.0	-	2	4	4	-	present	absent
0.36	1.11	0.04	0.12	100	120	8	10.5	-	2	2	1	1	0	-	-
0.38	1.04	0.34	0.93	120	240	8	9.5	-	1	3	2	-	2	present	absent
0.46	1.08	0.32	0.76	74	120	5	5.0	6.0	2	3	3	4	1	-	present
0.36	1.03	0.26	0.75	63	180	13	17.0	17.5	3	4	6	1	1	present	present
0.43	-	-	-	-	120	3	5.0	6.0	1	2	2	2	1	present	absent
0.34	0.97	0.32	0.92	121	120	4	7.5	8.0	2	2	3	3	0	present	absent
0.36	0.87	0.39	0.95	104	180	3	11.5	12.0	1	3	3	3	2	present	present
0.34	1.09	0.33	1.06	141	-	2	6.0	6.5	4	2	4	4	2	absent	present
0.38	1.02	0.29	0.76	120	120	-	6.0	8.0	4	4	4	4	0	absent	present
0.41	1.04	0.28	0.71	90	360	1	10.0	11.0	3	3	3	3	0	present	present
0.38	-	-	-	-	720	2	7.0	7.5	3	3	3	3	0	absent	absent
0.31	1.03	0.04	0.12	88	-	-	-	-	-	-	-	-	-	-	-
0.43	0.77	0.21	0.38	99	-	-	-	-	-	-	-	-	-	-	-
0.34	0.93	0.23	0.65	145	-	-	-	-	-	-	-	-	-	-	-
0.48	1.27	0.47	1.26	76	-	-	-	-	-	-	-	-	-	-	-
0.36	1.07	0.32	0.94	97	-	-	-	-	-	-	-	-	-	-	-
0.38	0.96	0.33	0.81	114	-	-	-	-	-	-	-	-	-	-	-

appendix 12

Courtship components of male offspring of seventeen wildtype *N. vitripennis* females

wildstrains	latency [sec]	hnd_1	h2-h1	cycle_1 [sec]	total no. of series	wildstrains	latency [sec]	hnd_1	h2-h1	cycle_1 [sec]	total no. of series	wildstrains	latency [sec]	hnd_1	h2-h1	cycle_1 [sec]	total no. of series	wildstrains	latency [sec]	hnd_1	h2-h1	cycle_1 [sec]	total no. of series
16/1	3,9	10	-	11,1	2	27/1	25,0	6	-2	7,0	6	24/10A	171,0	6	-2	5,4	6	10/8B	8,9	4	-1	4,3	5
16/1	135,4	2	5	7,1	5	27/1	46,8	6	0	6,9	4	24/10A	41,5	-	3	2,7	12	24/3B	5,9	4	-1	3,3	-
16/1	102,4	7	-1	7,7	4	27/1	65,9	4	-1	8,1	8	24/10A	28,6	3	-1	5,0	20	24/3B	68,3	3	-3	2,5	5
16/1	24,1	3	1	5,4	6	27/1	20,8	5	-1	6,6	6	24/10A	33,7	5	0	5,2	6	24/3B	36,0	5	-2	4,4	7
16/1	84,4	5	0	7,2	5	27/1	8,4	6	-1	7,5	5	24/10A	236,5	5	-1	4,6	8	24/3B	79,5	5	-2	5,3	10
16/1	99,4	7	-2	6,9	6	27/1	95,3	5	-1	6,8	5	24/10A	86,7	2	-1	4,7	6	24/3B	21,6	5	-1	5,2	9
16/1	65,4	3	2	5,5	5	27/1	88,9	8	-3	7,9	8	15/3b	82,3	7	0	5,3	7	24/3B	21,5	4	0	3,6	10
16/1	7,1	7	-2	8,6	3	27/1	84,2	-	-	-	4	15/3b	109,7	5	-2	9,2	7	24/3B	66,4	3	1	3,9	10
16/1	6,1	8	0	8,8	7	27/1	63,5	7	-2	8,0	9	15/3b	23,7	9	-3	5,8	8	24/3B	94,9	6	-2	5,1	8
16/1	87,1	7	-2	8,3	11	27/1	129,6	4	0	7,2	6	15/3b	50,1	7	-1	5,9	7	24/3B	65,3	4	0	5,0	11
22/1	170,4	8	-1	8,0	7	27/1	12,5	6	-2	6,6	10	15/3b	87,7	7	-2	6,9	14	24/3B	213,3	5	-2	4,4	13
22/1	88,5	5	1	16,5	4	27/1	75,1	5	-2	7,1	7	15/3b	59,9	3	0	6,3	10	17/1B	35,6	6	-1	5,2	10
27/1	20,6	8	-2	7,3	6	27/1	262,5	6	-1	7,5	7	15/3b	4,0	5	-1	6,5	13	17/1B	207,3	5	-1	7,4	9
27/1	34,8	10	-	8,5	2	27/7	83,0	5	-2	7,0	7	15/3b	21,4	7	-2	5,4	14	17/1B	64,2	6	-2	6,6	10
27/1	262,8	5	0	7,4	7	27/7	247,8	8	-3	8,4	5	15/3b	26,9	3	0	4,3	15	17/1B	261,6	6	-1	6,7	7
28/1	100,3	4	0	6,9	6	16/1B	79,4	3	-1	6,8	9	15/3b	156,9	7	-3	6,8	9	17/1B	22,5	4	-1	4,3	10
28/1	114,5	5	1	6,8	3	16/1B	77,9	4	-2	6,2	11	22/5A	19,0	4	0	4,7	6	17/1B	109,1	6	-1	5,8	8
22/1	38,5	5	-1	6,8	5	16/1B	19,2	4	-2	4,6	9	22/5A	119,8	7	-2	6,6	4	17/1B	31,5	5	-1	5,5	8
22/1	205,8	6	-1	8,5	8	16/1B	245,5	5	-2	6,5	11	22/5A	151,2	5	-1	6,6	6	17/1B	47,4	5	-1	5,9	8
22/1	23,0	5	-1	5,7	5	16/1B	54,5	3	2	4,5	6	22/5A	15,8	4	-1	5,2	7	17/1B	30,0	5	-1	5,1	8
22/1	88,0	6	-2	6,9	5	16/1B	32,9	3	0	5,4	13	22/5A	93,3	4	-2	5,5	8	17/1B	55,8	7	-3	6,6	6
22/1	37,0	1	-	7,0	2	16/1B	46,2	3	0	6,1	13	22/5A	111,3	7	-2	6,3	7	11/9B	71,6	5	-2	6,0	7
22/1	71,0	4	1	6,7	7	16/1B	73,7	3	0	4,8	14	22/5A	49,8	5	-1	7,3	7	11/9B	43,3	2	-1	5,4	8
22/1	84,3	6	-2	7,4	6	16/1B	78,3	1	2	4,9	12	22/5A	265,5	6	-3	5,1	7	11/9B	209,1	6	-2	8,1	7
22/1	56,7	4	0	6,6	8	16/1B	32,4	3	-1	4,9	7	22/5A	20,1	6	-2	5,5	7	11/9B	16,8	5	-2	5,8	7
22/1	88,5	5	-1	7,2	7	4/11 B	14,4	6	-1	5,0	6	22/5A	62,4	6	-2	5,3	7	11/9B	50,4	6	-2	6,8	6
22/1	16,8	4	1	6,0	7	4/11 B	146,8	5	-2	5,3	10	4/2B	14,3	5	-1	4,3	9	11/9B	102,1	4	-1	7,5	6
22/1	23,2	6	-2	6,7	7	4/11 B	38,8	6	-2	4,8	5	4/2B	56,6	6	-1	5,5	12	11/9B	45,6	4	-2	6,0	8
22/1	18,1	5	1	7,0	6	4/11 B	97,6	5	-1	5,1	9	4/2B	52,5	4	1	0,0	8	11/9B	235,3	2	1	5,8	8
22/1	38,2	3	0	5,7	6	4/11 B	14,5	4	-1	5,2	8	4/2B	32,2	6	-2	4,4	9	11/9B	118,7	6	-2	6,4	7
22/1	148,4	6	-2	7,6	5	4/11 B	17,2	4	-1	4,3	8	4/2B	75,5	8	-4	5,9	9	11/9B	8,5	5	-1	5,5	6
22/1	203,1	6	-2	9,1	6	4/11 B	69,4	6	-2	5,4	10	4/2B	47,7	3	0	4,8	8	11/9B	85,4	5	-1	6,4	9
22/1	26,5	5	-1	7,2	7	19/10A	287,7	5	-2	4,9	11	4/2B	6,0	3	-1	4,0	9	11/3A	203,2	5	-2	8,8	8
22/1	35,5	4	0	6,4	10	19/10A	102,2	4	0	4,4	10	4/2B	12,6	7	-3	5,5	7	11/3A	46,0	3	1	4,3	3
22/7	198,0	4	-	6,5	2	19/10A	19,8	6	-2	4,9	3	4/2B	18,2	6	-2	4,0	7	11/3A	24,9	6	-2	7,1	7
22/7	21,0	6	-2	6,9	6	19/10A	15,0	3	1	4,7	6	4/2B	56,2	7	-3	5,5	7	11/3A	26,5	3	-1	5,6	4
22/7	153,8	5	-	10,7	4	19/10A	27,6	6	-1	5,5	8	4/2B	46,6	5	0	5,9	5	11/3A	79,3	6	-3	8,4	6
22/7	36,3	5	-1	7,3	6	19/10A	76,1	6	-1	5,2	11	10/8B	18,8	5	-1	5,1	4	11/3A	29,2	4	-1	5,5	9
22/7	41,3	6	-2	8,2	7	19/10A	10,5	4	-1	3,6	10	10/8B	25,2	5	-2	4,9	9	11/3A	36,2	4	-1	5,3	8
22/7	31,1	4	0	6,8	7	19/10A	22,0	5	-2	4,7	8	10/8B	14,7	6	-1	5,5	6	11/3A	45,5	5	-2	5,9	7
22/7	37,3	7	-2	9,1	5	19/10A	25,7	3	1	4,3	3	10/8B	60,8	5	-1	5,3	3	11/3A	36,5	4	-2	5,0	7
22/7	39,1	8	-4	8,1	5	19/10A	129,2	2	0	4,1	12	10/8B	5,6	6	-1	5,2	5	11/3A	186,5	5	-2	5,1	7
22/7	15,3	3	-1	6,1	7	24/10A	26,9	3	-1	4,7	10	10/8B	20,4	3	-1	4,6	7	11/3A	113,6	6	-3	7,2	9
27/1	36,7	6	-1	6,7	6	24/10A	15,5	6	-3	5,3	8	10/8B	24,6	5	-2	5,3	7	15/10B	142,0	8	-1	6,5	9
27/1	65,2	6	-2	8,7	6	24/10A	9,3	5	-3	0,0	9	10/8B	40,3	8	-2	5,4	5	15/10B	44,2	4	1	6,5	4
27/1	59,1	5	-2	7,6	5	24/10A	15,6	5	-2	0,0	9	10/8B	30,3	5	-1	4,8	6	15/10B	87,6	5	-1	6,1	7
15/10B	30,6	6	0	9,3	6	15/10B	42,1	4	-1	2,3	12	15/10B	104,0	6	-1	7,0	8						
15/10B	32,9	8	-2	9,4	9	15/10B	137,4	4	0	6,9	7	15/10B	197,8	7	-1	7,7	8						

Lebenslauf

Persönliche Daten	Christof Pietsch Berliner Str. 4 35321 Laubach
	verheiratet, zwei Kinder
Schulausbildung	
Aug. 1975 - Juli 1979	Grundschule Laubach
Sept. 1979 - Juni 1985	Gesamtschule Laubach
Sept. 1985 - Juni 1988	Laubach Kolleg - Abschluss: Abitur
Berufsausbildung	
Sept. 1988 - Juli 1990	Zierpflanzengärtner, Botanischer Garten der Universität Marburg - Abschluss: Gärtner
Studium	Diplom Biologie
WS 90 - SS 93	Universität Giessen
WS 93 - WS 95	Universität Gesamthochschule Kassel
	Schwerpunkte: Zoologie, Botanik, Bodenkunde
Mai - Okt. 1995	Voruntersuchung zur Diplomarbeit
Feb. - März 1996	Mündliche Diplomprüfungen
Mai 1996 - Juli 1997	Diplomarbeit am Deutschen Entomologischen Institut, Eberswalde
	Thema: Pollenquellen und Neststandorte zwei syntoper Furchenbienen - Abschluss: Diplom Biologie, sehr gut
Beruflicher Werdegang	
Nov. 1997 - Okt. 1998	Geschäftsführer der Schutzgemeinschaft Vogelsberg e.V.
Nov. 1998 - Mai 1999	Wissenschaftliche Hilfskraft im BMBF-DLR Projekt "Araukarienwald" in Brasilien, Lehrstuhl für Entwicklungsphysiologie, Universität Tübingen
Juni 1999 - April 2000	Wissenschaftlicher Mitarbeiter im oben genannten Projekt
Mai 2000 - Okt. 2000	Vorübergehende Tätigkeit im Planungsbüro PNL, Hungen (Geburt des 1. Kindes)
Nov. 2001 - April 2002	Wissenschaftlicher Mitarbeiter im BMBF-DLR Projekt "Araukarienwald"
ab Mai 2002	Wissenschaftlicher Mitarbeiter am Lehrstuhl für Verhaltensphysiologie und Soziobiologie, Universität Würzburg

Veröffentlichungen

Pietsch, Rütten, Gadau (2004) Eleven microsatellite markers in *Nasonia*, ASHMEAD 1904 (Hymenoptera, Pteromalidae).
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Pietsch, van den Assem, Veenstra, van der Zande, Beukeboom and Gadau (2004) Genetic architecture of male courtship behavior in *Nasonia* (Hymenoptera, Pteromalidae) and hybrid effects in an interspecific cross. in Vorbereitung

Erklärung

Hiermit erkläre ich, die vorliegende Arbeit in allen Teilen selbständig und nur mit den angegebenen Hilfsmitteln und Quellen angefertigt zu haben.

Diese Dissertation hat weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegen.

Ich erkläre hiermit, früher weder akademische Grade erworben zu haben, noch habe ich versucht solche zu erlangen

Würzburg, den

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