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ORIGINAL ARTICLE

The genetic basis of interspecies host preference differences in the model parasitoid *Nasonia*

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The genetic basis of host preference has been investigated in only a few species. It is relevant to important questions in evolutionary biology, including sympatric speciation, generalist versus specialist adaptation, and parasite–host co-evolution. Here we show that a major locus strongly influences host preference in *Nasonia*. *Nasonia* are parasitic wasps that utilize fly pupae; *Nasonia vitripennis* is a generalist that parasitizes a diverse set of hosts, whereas *Nasonia girault*i specializes in *Protocalliphora* (bird blowflies). In laboratory choice experiments using *Protocalliphora* and *Sarcophaga* (flesh flies), *N. vitripennis* shows a preference for *Sarcophaga*, whereas *N. girault*i shows a preference for *Protocalliphora*. Through a series of interspecies crosses, we have introgressed a major locus affecting host preference from *N. girault*i into *N. vitripennis*. The *N. girault*i allele is dominant and greatly increases preference for *Protocalliphora* pupae in the introgression line relative to the recessive *N. vitripennis* allele. Through the utilization of a *Nasonia* genotyping microarray, we have identified the introgressed region as 16 Mb of chromosome 4, although a more complete analysis is necessary to determine the exact genetic architecture of host preference in the genus. To our knowledge, this is the first introgression of the host preference of one parasitoid species into another, as well as one of the few cases of introgression of a behavioral gene between species.
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Introduction

The genetic basis of host preference is relevant to a number of fundamental evolutionary questions. These include evolution of specialization and generalization (Whitlock 1996; Kelley and Farrell, 1998), sympatric speciation (Rice 1987; Dieckmann and Doebelli 1999; Kondrashov and Kondrashov, 1999), host shifts (Knowles *et al*., 1999; Groman and Pellmyr, 2000) and parasite–host and plant–herbivore co-evolution (Futuyma and Mitter, 1996; Forbes *et al*., 2009). For example, the presence of host choice can drive the evolution of specialization, as organisms adapt to the hosts to which they are more frequently exposed (Whitlock 1996; Kawecki 1998). In speciation models, the simpler the genetic basis of host preference and performance, and the more these traits are tightly linked, the more likely sympatric speciation is to occur (Fry 2003). An additional consideration is that parasitoids are widely used for the biological control of pests of agricultural importance (Quicke, 1997). A better understanding of the genetics of host range in parasitoids could also facilitate genetic improvement of these insects in biological pest control, by providing mechanisms for genetic manipulation of host usage.

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Host selection behavior involves several phases, including habitat location, host location, host recognition and host acceptance (Jaenike, 1990; Vinson, 1998). All these stages may be under both genetic and environmental influence (Geervliet *et al*., 1998). However, the genetic basis of these behaviors has been investigated in only a few systems. Most of the work on host selection behavior in arthropods has been done in phytophagous insects (reviewed by Jaenike, 1990) parasitic hymenoptera (reviewed by Vinson, 1998), and ticks and mites (Magalhães *et al*., 2007). Genetic studies have suggested a wide variation between systems in the number of loci and mode of inheritance involved in host preference (for example, Jaenike, 1987; Thompson *et al*., 1990; Keese, 1996; Messina and Slade, 1997; Tucic *et al*., 1997; Hawthorne and Via, 2001; Nylin *et al*., 2005).

The most well-studied genetic system for host preference is that of *Drosophila sechellia*, a species of *Drosophila* endemic to the Seychelles Islands which feeds solely on the fruit of *Morinda citrifolia*, which is toxic to most other *Drosophila* species (Louis and David, 1986; Jones, 2005). It was subsequently shown that two genes encoding odorant binding proteins affect the species' responses to hexanoic and octanoic acid, and therefore their attraction to the fruit (Matsuo *et al*., 2007). Knockout of one of these genes in *Drosophila melanogaster*, *Obp56e*, caused the flies to lose much of their aversion to morinda fruit (Dworkin and Jones, 2009). In aphids, Hawthorne and Via 2001) found a complex basis to host preference, with several groups of tightly linked quantitative trait loci involved in host choice and fitness.

The present work analyzes the genetics of host preference differences in the parasitic wasp *Nasonia*.

There are four species of *Nasonia*: *Nasonia vitripennis*, *Nasonia giraulti*, *Nasonia longicornis* and the newly described *Nasonia oneida* (Raychoudhury *et al*., 2010). The species are interfertile once cured of *Wolbachia* (Breeuwer and Werren, 1990, 1995), allowing traits from one species to be introgressed into another (Weston *et al*., 1999; Loehlin *et al*., 2010). With the recent sequencing of the genomes of three species (Werren *et al*., 2010) and a wealth of other resources becoming available (for example, Lynch and Desplan, 2006; Niehuis *et al*., 2010; Pannebakker *et al*., 2010), *Nasonia* provides a powerful system for studying the genetic basis of interspecies differences (Werren and Loehlin, 2009).

Nasonia consists of both a generalist and specialist species. *N. vitripennis* has a holarctic distribution and is a generalist that parasitizes a wide range of calyptrate flies, including blowflies, house flies and flesh flies. *N. giraulti* and *N. longicornis* specialize on bird blowflies (*Protocalliphora*, which *N. vitripennis* also parasitizes) and occur in northeastern and northwestern parts of North America, respectively (Darling and Werren, 1990). The newly described *N. oneida* also specializes in bird blowflies and is, at present, known to occur only in upstate New York, USA (Raychoudhury *et al*., 2010). *N. vitripennis*, *N. giraulti* and *N. oneida* occur microsympatrically in bird nests in eastern North America, whereas *N. vitripennis* is also found associated with carrion-breeding flies.

As host choice is hypothesized to drive the evolution of specialization (Whitlock 1996; Kawecki 1998), the genetics of host preference is particularly relevant to the evolution of host usage in *Nasonia*. Evidence of a host preference locus was originally detected during the introgression of male-specific wing-size locus $ws1_g$ from *N. giraulti* into *N. vitripennis* (Weston *et al*., 1999). Pure breeding of the line with $ws1_g$ in a largely *N. vitripennis* genetic background was difficult because homozygous females did not sting the *Sarcophaga* (flesh fly) hosts that are used for the maintenance of wasp strains in the laboratory and are regular hosts of *N. vitripennis* in the wild. A pilot experiment indicated that they did sting *Protocalliphora* hosts. Subsequently, $ws1_g$ was separated from these effects by recombination, allowing pure-breeding of $ws1_g$ (Weston *et al*., 1999). Here, we backcross the region around $ws1_g$ from *N. giraulti* into *N. vitripennis* using newly available visible markers, and map a major host-preference effect in the region.

Materials and methods

Nasonia strains and maintenance

The general biology of *Nasonia* is described by Whiting (1967). Cultures of *Nasonia* were maintained in the laboratory with constant light and temperature (25 °C) on *Sarcophaga* pupae. Under these conditions the generation time is approximately 14 days. For laboratory experiments on host preference and acceptance, the standard reference strains of *N. vitripennis* (ASymCx) and *N. giraulti* (RV2Xu) were used (Werren *et al*., 2010). To introgress (backcross) the region around $ws1_g$ from *N. giraulti* into *N. vitripennis*, we used the mutant *N. vitripennis* strain peach (*pe333*). Previous studies had revealed that this eye color mutant interacts epistatically with a natural eye color allele in *N. giraulti* (bk_g) that is linked to $ws1_g$, thus permitting easy tracking of the region during backcrossing.

For host preference experiments we used *Sarcophaga bullata* and *Protocalliphora sialia* pupae. A *Sarcophaga* culture was maintained in the laboratory, whereas *Protocalliphora* were obtained as larvae from bluebird and tree swallow nests during the summer months. The larvae were separated and allowed to pupate. Two days after pupation, both *Sarcophaga* and *Protocalliphora* were placed in a refrigerator at 4 °C, where they were stored for upto 4 weeks before their use in experiments.

Host-acceptance tests of field-collected wasps

To test for the acceptance of *Sarcophaga* hosts by field-collected wasps, we collected bluebird and tree swallow nests from eight different states in the eastern and midwestern parts of United States of America (New York, Ohio, Virginia, Pennsylvania, Indiana, Minnesota, Michigan and Wisconsin). Wasps were allowed to emerge from the nests in the laboratory, and we collected females from those nests that contained either all *N. giraulti* (14 nests) or all *N. vitripennis* (102 nests). To assess acceptance of *Sarcophaga* hosts, the females were placed in a vial with one *Sarcophaga* pupa and allowed to parasitize until the wasp dies (approximately 3–6 days). Two to three weeks later, hosts were scored for the presence of adult flies or wasps (adults or diapausing larvae).

Host preference and acceptance experiments

Observations were carried out to characterize the behavioral response of *Nasonia* strains to *Sarcophaga* and *Protocalliphora* pupae. Virgin females, 2–3 days old, were placed in individual vials. Each female was given either one *Sarcophaga* host and one *Protocalliphora* host in a host preference (that is, choice) experiment or two *Sarcophaga* in a host acceptance experiment. Vials were set horizontally so that the female's behavior could be observed. The female's contact with and stinging of hosts was recorded. A 'contact' was recorded if the female was on the host but not stinging it. A 'sting' was recorded if the observer could see the ovipositor probing/stinging the host. Observations were made every 5 min for the first hour, every 10 min for the second hour and subsequently every 15 min. Observations ceased approximately 4.5 h after the female was first given the host. Each wasp was then scored for (1) whether or not it contacted a host at all during observation (contact), (2) how much time it spent contacting each host (time spent on host) and (3) whether or not it stung during observation (stinging). After 24 h, each host was removed and scored two to three weeks later for the presence of adult flies or wasps (adults or diapausing larvae). Statistical comparisons were carried out using contingency χ^2 tests or Mann–Whitney U tests. Both host acceptance and preference tests were carried out on *N. vitripennis* (strains ASymCx and peach), *N. giraulti* (strain RV2Xu) and heterozygous $bkbw_g/+_v$ introgression females (described below).

Introgression of the $bkbw_g$ region into *N. vitripennis*

To test for host preference effects in the $ws1_g$ region, we introgressed (backcrossed) the region around *ws1* from *N. giraulti* into an *N. vitripennis* genetic background. We

hereafter refer to this region as $bkbw_g$ (for black eyes, big wings and naturally occurring *N. girault*i visible markers in the region). Specifically, the $bkbw_g$ region contains the visible markers $ws1_g$, sww_g, and bk_g (see bottom of Figure 1). These visible markers, described below in more detail, allow heterozygous $bkbw_g/+_v$ females to be visibly distinguished from homozygous $bkbw_g/bkbw_g$ females and $bkbw_g$ males to be visibly distinguished from $+_v$ males.

The process of the introgression is outlined in Figure 1. Initially, *N. girault*i females were crossed to *N. vitripennis* peach males (Figure 1, parental). F_1 hybrid females were then backcrossed to *N. vitripennis* peach males (Figure 1, backcross). Each backcross generation, hybrid heterozygous $bkbw_g/+_v$ females, identified using visible markers in the region (Figure 1), were mated to *N. vitripennis* peach males. After 10 generations, an attempt was made to produce a homozygous $bkbw_g$ strain by crossing $bkbw_g$ hybrid males to heterozygous $bkbw_g/+_v$ females (Figure 1, purebreed). In the next generation, all females were mated to $bkbw_g$ males. The incidence of failure to parasitize *Sarcophaga* hosts increased dramatically, and nearly all females parasitizing hosts proved to be $bkbw_g/+_v$ heterozygotes rather than $bkbw_g/bkbw_g$ homozygotes. This made the production of a purebred $bkbw_g$ introgression line impossible. It is to be noted that these hybrid incompatibilities are only seen in the purebreeding stage and not in the late-generation backcrosses, because hybrid incompatibilities in *Nasonia* tend to be recessive (Breeuwer and Werren, 1995). The $bkbw_g$ strain is therefore maintained heterozygously, by crossing heterozygous $bkbw_g/+_v$ females with $bkbw_g$ males.

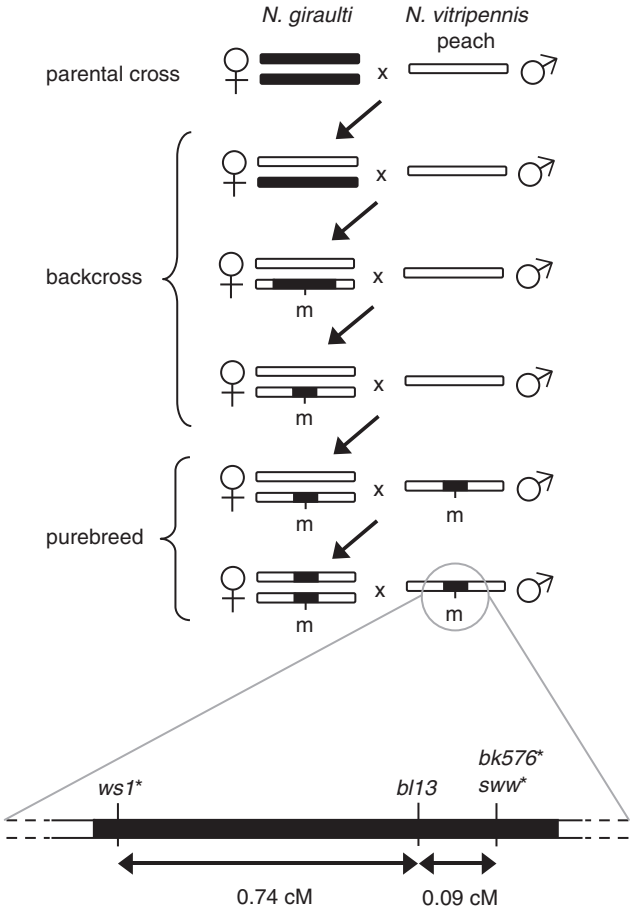

Figure 1 Generation of the $bkbw_g$ (for black eyes, big wings and naturally occurring *N. girault*i visible markers in the region) introgression line and map of visible markers in the region. In the parental cross, *Nasonia girault*i females were mated to *Nasonia vitripennis* peach males. In each backcross generation, heterozygous females, identified by visible markers (m) in the $bkbw_g$ region, were mated to *Nasonia vitripennis* peach males to further reduce the size of the introgression. To purebreed the line, heterozygous $bkbw_g/+_v$ females were mated to $bkbw_g$ introgression males, and their homozygous $bkbw_g/bkbw_g$ introgression female offspring were again mated to $bkbw_g$ introgression males in an attempt to produce an isogenic line. Inset is a map of the $bkbw_g$ region, including the location of visible markers *ws1* (*wing size 1*), *bl13* (*blue 13*), *bk576* (*black 576*) and sww (*shorter wider wings*). Markers indicated with asterisks were used to track the $bkbw_g$ introgression. Distances between visible markers are shown in centiMorgans (cM).

Visible markers in the $bkbw_g$ region

Integrity of the $bkbw_g$ introgression strain is maintained using visible markers (a map of these markers is shown at the bottom of Figure 1). The region maps onto linkage group IV (chromosome 4), and on one end lies the major male wing size QTL *ws1* (Weston *et al*., 1999, Werren *et al*., 2010). Approximately 0.74 cM from *ws1* is the mutant body color allele *bl13*, which causes a blue-colored body. This *N. vitripennis* mutation was originally generated by Saul *et al*. (1965), but had been mapped incorrectly to linkage group III (Saul *et al*., 1967). On the opposite side of *bl13*, 0.09 cM away, lay the loci *bk* and *sww*. The *bk* and *sww* loci were discovered during experiments conducted to introgress additional wing size QTL, using a *N. vitripennis* strain (peach) with the R-locus mutant *pe333* on chromosome 5 which causes 'peach' colored eyes (JH Werren and L Enders, unpublished data). These experiments revealed an epistatic interaction between *pe333* and *N. girault*i wild-type eye locus bk_g, which creates 'oyster'-colored eyes when both markers are homozygous. Epistatic effects among some eye color mutants generated in *N. vitripennis* had been previously observed (Saul and Kayhart, 1956), apparently involving mutants in the red versus brown pigment pathways. Subsequent genetic analyses revealed that the natural *N. girault*i eye color effect was allelic to the *N. vitripennis* locus *bk576* on linkage group IV. A mutant at *bk576* causes blackish eyes (Saul *et al*., 1965). Introgression males showing the oyster eye phenotype also had large wings, even larger than the standard $ws1_g$ males. The wing size effects within the region are due to introgression of *N. girault*i alleles at *ws1* and a second locus, called *shorter wider wings* (*sww*). Analyses of *sww* will be reported elsewhere.

Mapping of the $bkbw_g$ region using a genotyping microarray

To ascertain the size and content of the $bkbw_g$ region, we used a genotyping microarray that has been developed to genotype hybrids between *N. vitripennis* and *N. girault*i. Recent sequencing of the genome of *N. vitripennis* and partial sequencing of *N. girault*i (Werren *et al*., 2010) has identified an abundance of interspecies polymorphisms. *N. girault*i reads were aligned to the *N. vitripennis* genome sequence, and single-nucleotide polymorphisms, small insertions and

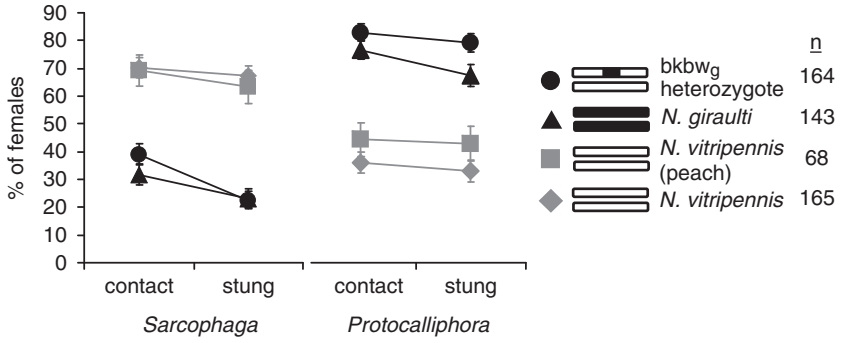

Figure 2 Behavior of wasps in host preference experiments. *Nasonia vitripennis* strains ASymCx and peach, *Nasonia girault* strain RV2Xu, and heterozygous bkbw$_g$/+$_v$ introgression females were tested. Genetic content of the wasps is shown in the chromosomes to the right, with white representing *Nasonia vitripennis* DNA and black representing *Nasonia girault* DNA. As can be seen, heterozygous bkbw$_g$/+$_v$ females contain a small region of *Nasonia girault* DNA in a largely *Nasonia vitripennis* genetic background. Wasps were given one *Sarcophaga* and one *Protocalliphora* host and observed for 4.5 h. The percent which contacted and stung each host is shown, and error bars indicate standard error of proportions (Sokal and Rohlf, 1969). Sample size for each strain is given to the right of the strain names.

deletions were used to design oligonucleotide probes to discriminate between *N. vitripennis* and *N. girault* DNA (Werren *et al*., 2010). Probes for >19 000 loci, covering 929 scaffolds and 86% of the assembled genome, were printed on custom NimbleGen microarrays (Madison, WI, USA). The details of the microarray will be described elsewhere (Desjardins CA *et al*., unpublished).

Deoxyribose nucleic acid was prepared from a single bkbw$_g$ introgression male using a Puregene Gentra DNA extraction kit (Qiagen, Valencia, CA, USA) using the protocol for a single *Drosophila* (http://www1.qiagen.com/Products/GenomicDnaStabilizationPurification/GentraPuregeneCellKit.aspx). This DNA was subsequently amplified via multiple displacement amplification using an Illustra Genomiphi V2 kit (GE Healthcare, Piscataway, NJ, USA). This DNA was labeled and hybridized to the array according to described protocols (Werren *et al*., 2010). After hybridization, each locus on the array was identified as *N. vitripennis*, *N. girault* or ambiguous. We then examined the loci in all major (>350 Kb) scaffolds (contiguous DNA sequences) on chromosome 4 to determine the genotypes of the scaffolds.

Results

N. vitripennis and *N. girault* differ in host preference

In order to assess differences in host acceptance between *Nasonia* species, *N. girault* and *N. vitripennis* wasps that had emerged from field collected birds nests were tested for acceptance rates of *Sarcophaga* pupae. Whereas 83% (2260 of 2739) of *N. vitripennis* females stung *Sarcophaga* hosts, only 45% (184 of 410) of *N. girault* females did so ($\chi^2_1 = 291$, $P < 0.001$). Therefore, field-caught *N. vitripennis* females are significantly more accepting of *Sarcophaga* hosts than are *N. girault*.

We next tested standard *N. girault* and *N. vitripennis* laboratory strains for host preference and acceptance. In preference tests, wherein wasps were provided with one *Sarcophaga* and one *Protocalliphora* host, *N. girault* showed a clear preference for *Protocalliphora*, whereas *N. vitripennis* showed a preference for *Sarcophaga* (see Figure 2). *N. vitripennis* was significantly more likely to both contact and sting *Sarcophaga* than *Protocalliphora* (contact: $\chi^2_1 = 9.5$, $P < 0.01$; stinging: $\chi^2_1 = 10.1$, $P < 0.01$), whereas *N. girault* had a significantly greater probability of contacting and stinging *Protocalliphora* hosts than *Sarcophaga* (contact: $\chi^2_1 = 14.4$, $P < 0.001$; stinging: $\chi^2_1 = 17.0$, $P < 0.001$). As can been seen in Figure 3 *N. vitripennis* also spent significantly more time on the *Sarcophaga* host than *N. girault* (Mann–Whitney *U*-test, $z = 8.0$, $P < 0.0001$), whereas *N. girault* spent significantly more time on the *Protocalliphora* host than *N. vitripennis* (Mann–Whitney *U* test, $z = 6.2$, $P < 0.0001$).

However, when the laboratory strains of both species were presented with two *Sarcophaga* hosts in acceptance experiments, both strains were highly accepting of *Sarcophaga* (84%, $N = 89$ for *N. vitripennis* and 78%, $N = 79$ for *N. girault*). There was no significant difference between the two strains relative to whether they contacted or stung the *Sarcophaga* hosts (contact: $\chi^2_1 = 0.20$, $P = 0.65$; stinging: $\chi^2_1 = 0.66$, $P = 0.42$), although *N. girault* spent significantly less time on the *Sarcophaga* host than did *N. vitripennis* (Mann–Whitney *U* test, $z = 2.8$, $P < 0.01$; see Figure 3).

Introgression of the bkbw$_g$ region into *N. vitripennis* shows girault-like preference

Next, we tested host preference and acceptance of the bkbw$_g$ introgression strain. Preliminary tests of bkbw$_g$/bkbw$_g$ homozygous females showed a complete failure to successfully parasitize *Sarcophaga* and *Protocalliphora* hosts in both the *Sarcophaga* and *Protocalliphora* host choice experiments and the two *Sarcophaga* acceptance experiments (data not shown). We therefore focused our host choice and acceptance experiments on bkbw$_g$/+$_v$ heterozygous females, and as an additional control, we tested the preference of mutant *N. vitripennis* strain peach (the genetic background of the bkbw$_g$ introgression line).

The peach *N. vitripennis* strain shows the same host preferences as the standard *N. vitripennis* strain, as it was not significantly different from non-mutant *N. vitripennis* strain ASymCx for any behaviors (contact: $\chi^2_1 = 0.64$, $P = 0.42$; stinging: $\chi^2_1 = 1.3$, $P = 0.25$; see Figure 2). Peach was significantly different from *N. girault* for all behaviors (contact: $\chi^2_1 = 21.8$, $P < 0.001$; stinging: $\chi^2_1 = 23.2$, $P < 0.001$; host stung first: $\chi^2_1 = 28.4$, $P < 0.001$). Therefore,

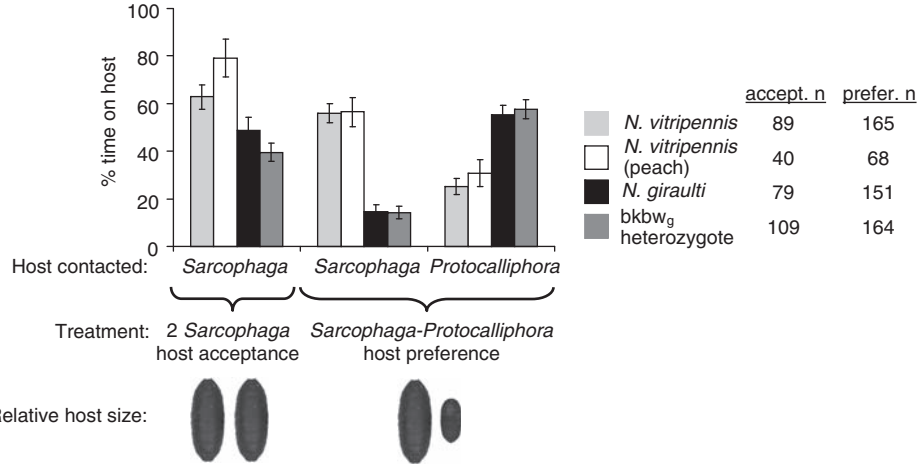

Figure 3 Time spent on hosts by wasps in host acceptance and preference experiments. *Nasonia vitripennis* strains ASymCx and peach, *Nasonia girault* strain RV2Xu, and heterozygous bkbw$_g$/+$_v$ introgression females were tested. Percent time is based on 28 observations over a 4.5 h period. Sample sizes for each strain in each experiment are shown to the right of the strain names. The relative sizes of *Sarcophaga* and *Protocalliphora* hosts are also shown.

it appears that the peach mutation (*pe333*) does not effect host preference in any significant way.

In contrast, heterozygous bkbw$_g$/+$_v$ females showed a *N. girault*-like host preference in all behaviors (contact: $\chi^2_1 = 0.31$, $P = 0.58$; stinging: $\chi^2_1 = 0.49$, $P = 0.48$; see Figure 2). Heterozygous bkbw$_g$/+$_v$ females were significantly more likely to contact and sting *Protocalliphora* than *Sarcophaga* in the 4.5 observation period (contact: $\chi^2_1 = 13.1$, $P < 0.001$; stinging: $\chi^2_1 = 27.6$, $P < 0.001$). Also, they spent similar amounts of time contacting each host as *N. girault* did (Mann–Whitney *U* test, time on *Protocalliphora*, $z = 0.9$ $P = 0.18$, time on *Sarcophaga*, $z = 0.7$, $P = 0.25$; see Figure 3). Heterozygous bkbw$_g$/+$_v$ females show a *N. girault*-like preference for *Protocalliphora* in a *N. vitripennis*-like genetic background, suggesting that the *N. girault* preference for *Protocalliphora* is dominant. The trait segregates in a Mendelian manner.

In the two *Sarcophaga* acceptance experiments, bkbw$_g$/+$_v$ heterozygous females did not show a significantly reduced contact rate relative to *N. girault* ($\chi^2_1 = 0.45$, $P = 0.42$), but they did show a significantly reduced stinging rate ($\chi^2_1 = 4.1$, $P < 0.05$). However, the stinging rate of the bkbw$_g$/+$_v$ heterozygous females was still relatively high (65%) and they did not spend significantly less time contacting hosts than *N. girault* (Mann–Whitney *U* test, $z = 1.4$, $P = 0.08$; see Figure 3), suggesting that only minor genetic incompatibility effects occur in bkbw$_g$/+$_v$ heterozygous females.

The bkbw$_g$ region maps to the centromeric portion of chromosome 4

We utilized the *Nasonia* genotyping microarray to genotype all scaffolds (contiguous DNA sequences) on chromosome 4 in a bkbw$_g$ introgression male (Table 1). For the majority of scaffolds, loci within a single scaffold were either scored as almost all *N. vitripennis* or almost all *N. girault*, allowing easy assignment of genotype to the scaffold as a whole. The exceptions to this were four scaffolds 23,29,40,52, in which each contain a region scored mostly as *N. vitripennis* adjacent to a region scored mostly as *N. girault*. Scaffolds 23 and 40 represent the outer bounds of the introgressed region. A few additional scaffolds, namely 26 and 133, were scored mostly as *N. girault*, but with a small number of internal consecutive loci scored as having a *N. vitripennis* genotype.

The bkbw$_g$ region maps to the central portion of chromosome 4 (markers 4.18–4.25 in Niehuis *et al*., 2010), encompassing 13 complete and 4 incomplete major (>350 Kb) scaffolds totalling 11 Mb. The region also contains approximately 29 smaller scaffolds, bringing the total number of scaffolds to 46 and the total size of the introgressed region to approximately 16 Mb, and is a region of low recombination. Included within the region appears to be a 4.5 Mb stretch of *N. vitripennis* DNA (Table 1). Various lines of evidence indicate that the bkbw$_g$ region spans the centromere (Werren *et al*., 2010). Although large, this region contains a wealth of visible and molecular markers, which can be used to fine-scale map the host preference allele.

Discussion

Nasonia girault shows a clear preference for *Protocalliphora*, the host genus it parasitizes in nature, over *Sarcophaga*. *N. vitripennis*, known to be a generalist from field studies, shows a preference for *Sarcophaga* in choice experiments. The host preference behavior of *N. girault* was introgressed into the genome of its sibling species *N. vitripennis*, along with chromosomal regions linked to the bkbw$_g$ loci. Our genetic analysis indicates one or more genes linked to the bkbw$_g$ region strongly influence host preference and that this effect segregates in a Mendelian manner. Females heterozygous for the bkbw$_g$ region show strong preference for *Protocalliphora* hosts with only minimal signs of reduced vigor, suggesting that a host-preference effect is present in the region independent of any hybrid viability effects. The preference is all the more remarkable, given that *Sarcophaga* hosts are much larger than *Protocalliphora* hosts (see Figure 3 for relative sizes), and therefore would be more likely to be encountered in the experiment. To our knowledge, this is the first report of the introgression of host preference from one parasitoid species into another one.

Table 1 Genotype of major scaffolds on chromosome 4 in a $bkbw_g$ introgression male

Scaffold	*Map location*	*Size (Kb)*	*No. of scored loci*			*Predicted genotype*
			Nasonia vitripennis	*Nasonia girauti*	*Ambiguous*	
4	4.01–4.15	5246	448	2	20	V
23	4.16–4.17	~519	31	0	7	V
	4.18–4.20	~1900	0	171	3	G
29	4.20	~200	0	13	0	G
	4.20	~1835	155	0	2	V
35	4.20	1595	142	2	6	V
108	4.20	481	23	2	1	V
52	4.20	~832	68	1	6	V
	4.20	~90	0	5	0	G
34	4.20–4.21	1326	0	127	0	G
26	4.21	1596	3	160	2	G
123	4.21	416	1	56	3	G
43	4.22	879	0	84	2	G
51	4.22	1217	2	124	3	G
66	4.22	619	0	51	0	G
82	4.22	492	1	30	2	G
109	4.22	483	0	33	1	G
130	4.22	385	0	42	0	G
133	4.22	387	4	38	0	G
143	4.22	399	0	38	2	G
77	4.23	479	0	32	0	G
40	4.25	~117	1	6	0	G
	4.25–4.29	~1760	183	0	10	V
9	4.29–4.41	4554	515	5	22	V

Abbreviations: $bkbw_g$, black eyes, big wings and naturally occurring *Nasonia girault*i visible markers in the region; G, *Nasonia girault*i; V, *Nasonia vitripennis*.
Locations of scaffolds are given as recombination-rate-based map markers from Niehuis *et al*. (2010), followed by size of the scaffolds or scaffold regions. The number of loci on the *Nasonia* genotyping microarray scored as *N. vitripennis*, *N. girault*i or ambiguous is also given, as is the predicted genotype of the scaffold or scaffold region. The $bkbw_g$ line contains two regions of *N. girault*i DNA separated by a 4.5 Mb stretch of *N. vitripennis* DNA, suggesting double recombination has moved *N. vitripennis* DNA back into the $bkbw_g$ region.

The preference for *Protocalliphora* seen in $bkbw_g/+_v$ heterozygous females also indicates the dominance of the *N. girault*i allele. Based on the results, we posit a host preference locus (*hp1*) within this region with preference for *Protocalliphora* dominant over non-preference. A pattern of dominance in the inheritance of oviposition preference has been found in several phytophagous insects (for example, Huettel and Bush, 1972; Jaenike, 1987; Keese, 1996), although all of these examples represent specialist versus specialist comparisons rather than the specialist versus generalist comparison done here. Additive genetic variance for oviposition preference has also been reported (for example, Schneider and Roush, 1987; Sheck and Gould, 1995; Tucic *et al*., 1997; Messina and Slade, 1997). If specialization in *Nasonia* is derived, a dominant allele could have rapidly swept through a diverging population.

As the $bkbw_g/+_v$ heterozygous females show host preference similar to *N. girault*i, it is then possible that host preference in *Nasonia* is controlled by a small number of loci or clusters of tightly linked loci. A moderate number of loci have been found controlling host preference in other insects; (Jones, 2005) found an oligogenic basis (intermediate genetic complexity) to preference of *Drosophila sechellia* for a chemical attractant (*Morinda* fruit toxin) present in their preferred host plant. In addition, (Hawthorne and Via, 2001) found four separate quantitative trace loci affecting host plant choice in host races of the aphid *Acyrthosiphon pisum*. This is directly relevant to some speciation models in which speciation is more likely when there are fewer loci controlling host preference (Fry, 2003).

For the evolution of specialization, differential performance on hosts is an important element in addition to differential preference. Although field-caught *N. girault*i showed highly reduced acceptance of *Sarcophaga* hosts, the laboratory strain RV2Xu did not. This is not unexpected, as *N. girault*i does not appear to parasitize *Sarcophaga* in the wild, but the standard strain has been reared in the laboratory on *Sarcophaga* for several years (*Protocalliphora* cannot be reared in the laboratory). Therefore, it is possible that *N. girault*i laboratory strains such as RV2Xu have developed an increased acceptance of *Sarcophaga* hosts. Clearly, however, this increased acceptance has not resulted in a preference for *Sarcophaga* over *Protocalliphora* in *N. girault*i laboratory strain RV2Xu. This suggests that loci controlling preference and acceptance (that is, performance) may be unlinked, a requirement for some speciation models (Bush, 1975; Fry, 2003). However, a change in host acceptance could be because of genetic or environmental causes, and further studies are needed to determine the genetic basis of host acceptance differences in *Nasonia* species.

The results presented here indicate that a host preference gene is linked to the $bkbw_g$ locus, encompassed by 16 Mb of *N. girault*i DNA around the centromere of chromosome 4. Within the introgressed $bkbw_g$ region appears to be a 4.5 Mb stretch of *N. vitripennis* DNA (Table 1). This may be the result of double recombination moving *N. vitripennis* DNA back into the introgressed region, possibly due to a gene in the region having a strong hybrid incompatibility effect. For example, the failure of $bkbw_g/bkbw_g$ homozygous females to parasitize any tested hosts may be indicative

of *N. girault*i alleles linked to the larger bkbw$_g$ region that cause genetic incompatibilities when homozygous in an *N. vitripennis* genetic background. It is also possible that the bkbw$_g$ region is contiguous and the region in question actually belongs to a different region of the genome, but has been placed here by a combination of assembly and mapping errors. A few internal scaffolds also contained small stretches of *N. vitripennis* DNA. Given the low-recombination rate in the region, it is unlikely that these regions are the result of double recombination moving *N. vitripennis* DNA back into the bkbw$_g$ region and more likely that they are misassembled and actually belong in other parts of the genome, as only a single assembly error is required to explain each of these regions. It is now necessary to partition the region by recombination to produce more targeted introgressions of the host preference allele, and this work is underway. This is being accomplished using mapping resources available for *Nasonia* (Niehuis *et al*., 2010; Werren *et al*., 2010) and several visible markers present within the region (Figure 1).

The presence of a *N. girault*i preference allele allows us to make some inferences on the evolution of host preference in *Nasonia*. It suggests that the transition between generalist and specialist strategies in *Nasonia* was not only an expansion or contraction of host range, but also included an actual change in preference for *Protocalliphora* hosts parasitized by both specialists and generalists. It is unknown whether either changes in host range or changes in host preference occurred first or whether they occurred simultaneously. Given that *Trichomalopsis*, close relatives of *Nasonia*, are largely generalists (Gibson and Floate, 2001), it is likely that generalism was the ancestral state for *Nasonia*, with subsequent evolution of specialization on *Protocalliphora* in the common ancestor of *N. girault*i, *N. longicornis* and *N. oneida*.

The evolution of oviposition preference is considered to be one of the driving forces in the divergence of phytophagous insect populations (Futuyma, 1987; Thompson, 1993). Similar views have been presented for the divergence of parasitic *Hymenoptera* (Godfray, 1994). In parasitoids, the differential usage of hosts may produce assortative mating as a pleiotropic consequence of female oviposition behavior. As *Nasonia* species mate locally on patchily distributed hosts and routinely inbreed (Drapeau and Werren, 1999), host preference differences might quickly lead to assortative mating. It is interesting to note that *N. girault*i shows a high propensity to mate within the host (Drapeau and Werren, 1999), producing a strong coupling of host preference and assortative mating. Therefore, it is possible that a shift in host preference was coupled with speciation events and perhaps with genetic bottlenecks because of the host shift. The low levels of genetic variation observed within *Nasonia* species is consistent with this scenario (Raychoudhury *et al*., 2010).

Conclusions

The results clearly indicate a major host preference locus (or set of tightly linked loci) in the region around bkbw$_g$. The *N. girault*i allele segregates in a Mendelian manner and imparts a dominant preference for *Protocalliphora* hosts in an otherwise *N. vitripennis* genetic background. Utilizing the *Nasonia* genotyping microarray, we have mapped the host preference effect to 16 Mb of chromosome 4. Fine-scale mapping of the host preference locus can now proceed, utilizing the wealth of mapping and molecular resources becoming available for *Nasonia* (Werren *et al*., 2010). To our knowledge, this is the first introgression of a host preference locus from one parasitoid species into another. Furthermore, this work represents one of the few examples of introgression of behavioral genes between species.

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