INTRASPECIFIC VARIATION IN SEXUAL ISOLATION IN THE JEWEL WASP NASONIA

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Abstract.—Divergence in mate recognition systems can lead to reproductive isolation. In this study, we investigate patterns of intraspecific variation that contribute to premating isolation within and between two haplodiploid species, Nasonia vitripennis and N. longicornis. In a broad-scale survey of 17 North American isofemale lines encompassing the two species, we report strong asymmetric sexual isolation between species and a dramatic level of intraspecific variation for mate discrimination between species. A general lack of incipient speciation was found, with the exception of low levels of interpopulational sexual isolation within N. vitripennis. Regression analysis shows that the degree of intraspecific variation for within-species mating frequency is not associated with the degree for between-species mating frequency. Reinforcement or reproductive character displacement may be involved in some of the variation in inter-species premating isolation.

Key words.—Behavior, premating isolation, reinforcement, sexual selection, speciation.

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Intraspecific variation in sexual isolation may signify the early stages of speciation. Such variation can take two forms: (1) intraspecific variation in reproductive isolation between populations within a species (incipient speciation); (2) intraspecific variation in reproductive isolation to closely related species (variable interspecific isolation). Studies of sexual isolation have characterized both patterns of intraspecific variation (Patterson and Stone 1952; Littlejohn and Loftus-Hills 1968; Zouros and D’Entremont 1980; Krebs 1990; Markow 1991; Wu et al. 1995; Miller et al. 1998). The latter pattern is often studied in the context of Dobzhansky’s reinforcement hypothesis, which proposes that natural selection will increase levels of prezygotic reproductive isolation between populations that produce unfit hybrids (Dobzhansky 1940). A by-product of reinforcement is that sympatric populations will show stronger levels of mate discrimination than allopatric populations of the same taxa (Noor 1995; Saetre et al. 1997; Rundle and Schluter 1998). Alternatively, sexual selection can generate locally adapted mating signals within populations. Divergence between populations could then lead to intraspecific variation for within and/or between species sexual isolation.

Sexual isolation (e.g., divergence in mate recognition systems) can arise due to sexual selection (West-Eberhard 1983; Kirkpatrick 1987; Holland and Rice 1998), genetic drift (Mayr 1963; Lande 1981), and natural selection (Fisher 1930; Dobzhansky 1937). In this study, we conduct a broad-scale survey of sexual isolation within and between two sibling species of the genus Nasonia. Our objectives are to determine the levels of variation in intra- and interspecific sexual isolation. The variation uncovered can then be used in genetic and population genetic experiments to investigate the evolution of premating isolation.

Nasonia is a complex of three closely related sibling species of haplodiploid parasitic wasps. Nasonia vitripennis (NV) exists worldwide and is a parasite of cyclocyrraphous flies that are found at carcasses and bird nests. Nasonia gi-
ations of laboratory rearing prior to experiments. The strains were removed from diapause and antibiotically cured of their Wolbachia infection with rifampicin treatment for three sequential generations. Curing of the Wolbachia infection was confirmed using a polymerase chain reaction (PCR) assay with primers previously described (Perrot-Minnot et al. 1996). Strains were then maintained in the laboratory for approximately 12 additional generations prior to the experiment. To test for mate discrimination, strains were divided into two groups: group I consisted of five NV strains and four NL strains; group II consisted of five NV and five NL strains. Two strains (NV Utah and NL Utah 1) were used in both groups.

The following strains were used (an abbreviated name for each strain is indicated in parentheses): group I: N. vitripennis: RNVMN 204K (NV Minnesota 1), RNVIN 217K (NV Indiana), RNVXIDB 431E (NV Idaho), RNVP A 2330 (NV Pennsylvania), RNVXUTC 406X (NV Utah); N. longicornis: RNLCA 9304 (NL California), RNLIDB 418E (NL Idaho), RNLNV 202H (NL Nebraska 1), RNLUTN 308V (NL Utah 1); group II: N. vitripennis: RNVMN 206Bi (NV Minnesota 2), RNVXNVB 403D (NV Nevada), RNVWYC 400G (NV Wyoming), RNVOH 204 (NV Ohio), RNVXUTC 406X (NV Utah); N. longicornis: RNLUTB 303–7L (NL Utah 2), NLUTB 305–3D (NL Utah 3), N LUTN 310 (NL Utah 4), RNLUTN 308V (NL Utah 1), RNLNV 206H (NL Nevada 2).

Strains were chosen from a broad geographic distribution, ranging from Pennsylvania to California for NV and NL. NV is cosmopolitan, being found throughout North America and the world, whereas NL is localized in western North America (Darling and Werren 1990).

Mating Tests

To assay variation in premating isolation, we tested male and female mating success in no-choice mating tests. Mating trials were designed as follows. Males and females of each strain were collected as virgin pupae approximately one day before eclosion. Pupae were sorted by gender into glass vials and kept at 25°C before eclosion. Mating frequency was asayed according to the methods below, using one- to two-day-old adults.

In each group, all possible crosses were performed in a diallel or grid format. Per replicate, two males and five females were placed in 12 × 75-mm glass vials at room temperature (25°C). After two hours, males were discarded and females were placed in individual vials. They were each immediately given one Sarcophaga bullata fleshly host. Female wasps were allowed to parasitize these until their death. Progeny were scored for presence or absence of females. Due to haplodiploidy, production of diploid females indicates that mating and fertilization occurred in Nasonia. Unmated females produce all-male families. Thus, the assay is designed to detect differences in mating behavior, rather than mechanical isolation or sperm-egg incompatibilities.

Validity of the Mating Assay

Tests were performed to determine the validity of using female (daughter) production as an assay for mating. Virgin males and females were singly paired in a glass vial and observed for 30 min. Of those replicates in which copulations were observed, females were subsequently hosted and the presence/absence of daughters was scored for each family. A total of 142 intraspecific matings were observed (involving two NV and two NL strains). Of these, all produced female offspring. Of 87 interspecific matings observed (involving the same strains), 86 produced female progeny, whereas of 32 cases where no mating occurred, none produced female progeny (i.e., all males were produced). These findings establish the validity of using daughter production as an assay for mating.

Data Analysis

Percent mating was scored based upon the production of female progeny in a cross. In haplodiploids, mated females produce female (diploid) and male (haploid) progeny, whereas unmated females produce only male progeny. Mating was therefore scored (indirectly) by analyzing the percentage of parental females that produced daughters among their progeny. All statistics were performed with MINITAB 11. Analyses of variance were performed on mating percentages that were arcsine transformed according to Sokal and Rohlf (1981). All pairwise comparisons were conducted with proportion tests. Regression analyses were conducted and associations were plotted linearly.

Results

Mating in Intra- and Interspecific Crosses

For the first group of strains (group I), all possible pairwise crosses were performed between five isofemale NV lines and four isofemale NL lines spanning nine different North American populations. Summary data of overall specieswide mating frequencies are shown in Figure 1. As expected, intraspecific matings occur readily in comparison to interspecific matings (93.4% vs. 39.1%). In interspecific crosses, sexual
isolation is strong, but not complete. There is a highly significant asymmetrical premating isolation pattern: NV male \times NV female matings occur more readily (59.2%) than NV female vs. NV male strain nested within the cross direction (Table 2). Analysis of variance on the premating asymmetry (and thus interspecific mating frequency) shows that female strain contributes significantly to the observed variation in interspecies mating frequency, whereas male strain does not (Table 1). Female strain accounts for 33.9% of the variation in interspecies matings, whereas male strain only accounts for 4.6% of the variation. These results support the view that differences in female preference govern the variation in male mating success in heterospecific matings, whereas NV male strain does not have a significant effect in any of the four cross directions (Table 3). NL female strain does have a highly significant effect on the observed mating success, whereas NV female strain does not (Table 3). For instance, NL female strain explains 58.7% and 85.6% of the variation in intra- and interspecific crosses, respectively.

The level of interspecific mating frequency differed significantly between strains. Results were most dramatic in analyses of interspecific mating frequency of NL females. A 50-fold difference in NL female interspecific mating frequency exists between Idaho females (52.2%) and California females (0.9%; Table 2). To confirm this extraordinary difference in NL female heterospecific mating frequency, two additional experiments were conducted. Following the previous design, NL Idaho and NL California females were crossed to NV Idaho males in both one- and two-hour assays. The results, which are shown in Table 4, confirm the variation initially described in Table 2. In the one-hour assay, NL Idaho females mated with NV Idaho males in 20.0% of the replicates, whereas NL California females did not mate.
with NV Idaho males at all (0.0%; Table 4). These percentages differ from the original two-hour experiment, but show the same trend in mate acceptance. A replicate experiment of the two-hour assay reconfirms the large difference in interspecific sexual isolation between these strains. Mating frequencies when NV Idaho males are crossed to NL Idaho and NL California females are 57.0% and 2.6%, respectively (Table 4).

We also analyzed the data to look for evidence of incipient sexual isolation by comparing the mating frequencies of within-strain (self) and between-strain within species (nonself) crosses. Figure 2 shows that for both species, nonself crosses yield lower mating percentages than self crosses, but the finding is only significant for NV ($P < 0.01$). For instance, crosses with either NV Minnesota 1 and NV Indiana males to NV Utah females show significantly reduced mating frequencies, 81.8% ($n = 22$) and 87.5% ($n = 16$), in comparison to the self-crosses, 100.0% ($n > 16$, $P < 0.05$).

A second set of crosses (group II) yield generally similar results. Crosses were performed with a different set of strains, except NV Utah and NL Utah 1 are common to the two groups. All possible pairwise crosses were set up with five NV and five NL isofemale lines. Summary data of overall interspecific mating success are shown in Figure 1. Data show that interspecific matings occur readily in comparison to interspecific matings. A highly significant premating isolation asymmetry was again found in the same direction as that of group I: NL male $\times$ NV female matings occur more readily than NV male $\times$ NL female matings (Table 1, $F = 149.39$, $P < 0.001$). Female strain had a marginally significant effect on the mating asymmetry ($8.3\%$ of the variation), whereas male strain did not ($4.9\%$ of the variation). The majority of the variation ($71.5\%$) is due to the species (i.e., cross direction). The finding differs from group I data, in which female strain explained a larger proportion of the variation. This difference may be attributed to reduced genetic variation among the group II strains, because four of the five NL lines are from the same state (Utah).

Table 5 shows patterns of male and female mating success across all possible group II crosses. To determine levels of intraspecific variance in mating frequencies, an analysis of variance was conducted within each of the four species-wide cross directions. Male and female strain were included as factors. While there is a moderate range of variation in male mating success in heterospecific matings, male strain tends not to have a significant effect in any of the four cross directions (Table 3, Two-way ANOVA, $P > 0.05$). On average, the range of male mating success variation in heterospecific crosses is greater for NL males (67.2% to 93.1%) than for NV males (18.9% to 33.3%). NL female strain does have a significant effect on the observed mating frequency variation, while NV female strain does not (Table 3).

Intraspecific variance in interspecific mating frequency was again revealed in our analysis. In contrast to group I, the greatest range of female mating frequency occurs within NV. For example, the frequency of NV Ohio females that mated with NL males is 65.1% versus 90.3% for NV Minnesota 2 females (Table 5). Interestingly, NV Minnesota 2 females mate with NL males almost as readily as they do with NV males (99.0%). Such female strain variation in interspecific mating frequency suggests that female preference may be actively evolving within local populations of NV. The Minnesota population is different from others in that NV was not found microsympatrically with either NL or NG, in contrast to all other populations tested (unpubl. data).

To detect patterns of incipient sexual isolation, mating fre-
Intraspecific Variation in Sexual Isolation

Fig. 3. Linear plots of interspecific female mating frequency regressed against intraspecific female mating frequency. Each circle/diamond denotes a geographic strain. Solid black circles denote data for *Nasonia vitripennis* and unfilled diamonds for *N. longicornis*. (A) Intraspecific mating frequency is calculated as the nonself mating frequency. (B) One outlier was excluded from the analysis and relative intraspecific mating frequency is calculated as the ratio of nonself mating frequency to self mating frequency.

Variance in Interspecific Mating Frequency: The Role of Sexual Selection

A regression analysis was conducted to make statistical inferences on the role of sexual selection in the observed intraspecific variance in interspecific mating frequency. The reasoning is as follows. If the variation in interspecific pre-mating isolation is due to incipient divergence between populations (e.g., sexual selection), then the level of premating isolation within species may be associated with the level of premating isolation between species. Assuming the targets of sexual selection (e.g., pheromones, courtship displays) are the same in both closely related species, subtle differences in mate discrimination between populations within a species could be expressed as much larger effects on interspecific isolation. One prediction follows: the strains showing a higher degree of intraspecific discrimination are most likely to show higher levels of interspecific discrimination. A regression analysis on female mating success was conducted for both NV and NL. In Figure 3A, interspecific mating frequency is regressed against intraspecific (nonself) mating frequency. No association is found for NV ($R^2 = 0.004, P = 0.858$), and a positive but nonsignificant association is found for NL ($R^2 = 0.329, P = 0.106$). Similarly, in Figure 3B, interspecific mating frequency is regressed against relative intraspecific mating frequency (calculated as the ratio of nonself mating frequency to self mating frequency), a measure that eliminates the confounding effects of mating propensity. No significant association was detected for NV ($R^2 = 0.133, P = 0.334$ and NL: $R^2 = 0.028, P = 0.692$). Furthermore, NV Utah, an NV strain with the highest level of self versus nonself intraspecific mating, had typical levels of female mating frequency with NL males (60.3%). Similarly, the NL California strain, which rarely mated with NV males (0.9%) showed no evidence of intraspecific discrimination. Based on the data here, we conclude that the variation in interspecific mating success is not due to divergence in intraspecific mating success between populations. Therefore, there is no evidence that the inter- and intraspecific behavioral patterns share a common cause.

Discussion

The biological species concept defines species as populations that do not typically interbreed and are thus reproductively isolated (Mayr 1940). The widespread acceptance of Mayr’s concept had a major impact on speciation studies. The study of the origin of species became the study of reproductive isolating factors (Coyne 1992). For practical purposes, most studies have investigated strongly expressing isolating mechanisms that are fixed between divergent groups. However, such systems are most likely beyond the incipient stages of speciation.

Studies of intraspecific variation in reproductive isolation have been conducted in systems such as fruit flies (Patterson and Stone 1952; Krebs 1990; Noor 1995), mosquitoes (Laven 1959), flour beetles (Wade and Johnson 1994; Wade et al. 1995, 1997), wolf spiders (Miller et al. 1998), and treefrogs (Littlejohn and Lofus-Hills 1968). The present study concerns intraspecific variation in reproductive isolation in the haplodiploid jewel wasp *Nasonia*.

We found significant variation in sexual isolation both within and between species. However, the variation detected in between-species mating trials was much greater than the variation detected in mating trials between geographic populations of the same species. In fact, intraspecific trials with only one group of strains showed significantly higher mating frequencies in within-strain to between-strain crosses, and only in one species (NV). Thus, we found relatively little evidence for interpopulational premating isolation within the two species.

In contrast, a large range of within-species variation in intraspecific sexual isolation was uncovered. Both species showed dramatic differences across geographic populations in mating frequency from heterospecific crosses. The greatest range of variation was seen between female strains, rather...
than male strains (Tables 3, 4). In part, this suggests that female preference may be actively evolving in localized populations. There are a few a priori reasons why this is to be expected in Nasonia. First, females determine whether copulation occurs in Nasonia; forced copulations are not possible in these species (Assem and Werren 1994). For any mating to occur, the female must be stimulated to a state of receptivity by the male. Second, females rarely mate twice, whereas males will mate repeatedly; thus, there may be stronger selection on females to be “choosy” about their mates. Third, selection against maladaptive hybridization could drive the evolution of mate discrimination (e.g., reinforcement) in regions of sympatry.

The view that interspecies mating can lead to reinforcement of premating isolation is still controversial, although the possibility has gained some recent theoretical (Liou and Price 1994; Kelly and Noor 1996; Kirkpatrick and Servedio 1999) and empirical (Noor 1995; Saetre et al. 1997; Rundle and Schluter 1998) support. There are several reasons for why reinforcement might be occurring in natural populations of Nasonia. First, there is potential for hybridization between the species in nature. NL and NV can co-occur microsympatrically, often in the same bird nests where hosts occur (Darling and Werren 1990; J. Werren, unpubl. data). Thus, in areas of sympatry and in the absence of mate discrimination, hybridization would be a frequent occurrence. Second, interspecific hybridization is maladaptive in Nasonia. Wolbachia-induced cytoplasmic incompatibility (CI) acts as a primary postzygotic isolating barrier between all three Nasonia species; CI can completely prevent hybrid production between the species (Breeuwer and Werren 1990; Bordenstein and Werren 1998; S. R. Bordenstein, J. A. J. Breeuwer, and J.H. Werren, unpubl. data). Even in the absence of Wolbachia-induced cytoplasmic incompatibility, other postmating incompatibilities exist (Breeuwer and Werren 1995) that could drive the evolution of premating isolation.

Although reinforcement was not the main subject of these experiments, some of the data are consistent with the theory. First, the interspecies premating asymmetry is in the expected direction (Watanabe and Kawanishi 1979): NL is more discriminatory than NV. There is likely to be an asymmetry in exposure to hybridization because NL is embedded within the geographic distribution of NV (Darling and Werren 1990). Additionally, NV is a generalist parasitoid found on a wider range of hosts and apparently at much higher population densities than NL. NL specializes on blowfly pupae inhabiting bird nests (Darling and Werren 1990; J. Werren, unpubl. data). Thus, NL likely experiences relatively higher incidences of microsympatry (i.e., occurring in same bird nests with NV) than NV.

A second line of evidence for reinforcement comes from patterns observed in particular crosses. NV female mating frequency in group I interspecific crosses varies widely across geographic regions (Table 2). In particular, NV Minnesota female mating frequency is high in matings to NL males (~88%), whereas the other NV strains show a significantly stronger degree of sexual isolation (~40–60%). The finding is notable, because the NV Minnesota strain is the most “allopatric-like” strain in our analysis. The other NV strains either occur sympatrically with NL in the western United States or with NG in the northeastern United States (Darling and Werren 1990). The same trend is observed in the group II dataset with a different NV Minnesota strain, although the differences are not to the same degree (Table 5). Future work will focus on a more systematic investigation of the possible role of reinforcement in the evolution of premating isolation.

Indirect evidence from NV and the eastern North American species N. giraulti (NG) is also consistent with reinforcement (or reproductive character displacement). Most parasitic wasps mate outside the host following emergence (but see Suzuki and Hiehata 1985). However, NG females have a high propensity to mate within the host prior to emergence, and thus often mate with siblings (Drapeau and Werren 1999). NG occurs microsympatrically with NV in bird nests over much of its range, and it has been hypothesized that within-host mating has evolved as a hybridization escape mechanism from the more common NV species. Nevertheless, the suggestion of reinforcement of premating isolation in Nasonia remains largely anecdotal at this time.

An alternative model for the evolution of a mating asymmetry has been posited by Kaneshiro (1983). According to this model, females from the ancestral lineage discriminate against males from the derived lineage, due to a loss of male mating components associated with a founder event in the derived lineage. Our data are inconsistent with this model, because the species showing more discrimination (NL) is the derived lineage (Campbell et al. 1993).

Sexual selection operating within populations could generate variation in interspecific mating, because subtle differences in interpopulational mate discrimination governed by sexual selection may be amplified in interspecific crosses. According to this view, the variation in interspecific mating is merely a by-product of sexual selection within species, rather than a character directly under natural selection (e.g., reinforcement). One prediction of the model is that the strains showing a higher degree of intraspecific discrimination are most likely to show high levels of interspecific discrimination. This was not the case: We failed to detect an association between intra- and interspecific female mating frequency for either NV or NL (Fig. 3). The finding suggests that divergence in interspecific mating is not due to divergence in intraspecific mating (e.g., via sexual selection). However, sample sizes are insufficient to confidently rule out this effect.

The availability of visible and molecular markers in the Nasonia complex (Gadau et al. 1999) will provide tools for a more detailed genetic analysis of premating isolation in Nasonia. Future work will focus on the genetic and ecological basis of large differences in mate discrimination in Nasonia.

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