

ASSORTATIVE MATING IN THE JEWEL WASP: 1. FEMALE MATCHING OF EYE-COLOR GENOTYPE, NOT HOST-FEEDING PHENOTYPE

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ABSTRACT

An experiment was performed to test the influence of two factors on assortative mating in the Jewel wasp (*Nasonia vitripennis*): (1) inherited eye-color genotype, and (2) conditioned foraging phenotype. To determine the female choice of sires, genetically marked wasps were used of two recessive mutant eye-color genotypes: white or red. Each female (of independently crossed eye-color and juvenile host) was given a choice of two males, one of each eye-color, counterbalanced for juvenile host: blowfly (*Sarcophaga bullata*) or housefly (*Musca domestica*) pupae. Blowfly-reared females produced more total offspring, indicating higher fertility. Red-eyed females produced more offspring sired by red-eyed males, indicating assortative mating by eye color. Blowfly-reared females, however, did not produce more offspring sired by blowfly-reared males, indicating no assortative mating by juvenile host.

INTRODUCTION

The Jewel wasp (*Nasonia vitripennis*) is a beneficial parasitoid insect which may feed on a variety of species of two-winged flies (*Diptera*), such as blowflies (*Sarcophaga bullata*) and houseflies (*Musca domestica*). Like certain other insects, the Jewel wasp is behaviorally predisposed in adulthood to feed its own offspring (by selective oviposition) on the particular host species that it fed on as a juvenile, or larva (Figueredo 1987, 1989). For such insects, it has been proposed that such behavioral feeding traditions may lead to mutual reproductive isolation between divergent feeding types, and thus to sympatric speciation, which is the splitting of one parent species into two or more daughter species without the benefit of geographical isolation (*e.g.*, Bush 1969, Futuyma and Mayer 1980, Berlocher and Feder 2002). In fact, host-specific reproductive isolation has been specifically proposed as a potential mechanism for sympatric speciation in the Jewel wasp (Smith and Cornell 1979).

In the absence of geographical barriers, reproductive isolation can only occur by some form of assortative mating. Assortative mating is the tendency for phenotypically similar individuals to mate. This tendency may be based on either one or several phenotypic variations and may include both physical characteristics and behavioral characteristics. Assortative mating can be distinguished from inbreeding in that inbreeding involves mating with individuals which are genetically similar at all or most variable loci whereas assortative mating minimally involves similarity only at those loci affecting variation in the traits for which the assortment occurs. In nature, most assortative mating is positive, favoring similarity over dissimilarity (Partridge 1983). Although positive assortment can theoretically lead to sympatric speciation (Bush 1969, Tauber and Tauber

1977a, 1977b), this potential consequence is believed to be epiphenomenal and not is hypothesized to represent its evolved adaptive function.

Assortative mating may be passive or active. *Passive* assortative mating can occur as a result of any persistent habitat preferences among individuals with different host-plant feeding experience that might cause them to breed in the same habitats in which they developed (Papaj and Prokopy 1988, Feder et al. 1994), as in the case of the Apple Maggot fly (*Rhagoletis pomonella*). This habitat preference might be mediated by differential attraction to volatile host-specific semiochemicals, as in the case of the Apple Maggot Fly (Forbes et al. 2005), the Apple fruit moth (*Argyresthia conjugella*) (Bengtsson et al. 2006), and the pea aphid (*Acyrtosiphon pisum*) (Del Campo et al. 2003). Segregation of host-specific phenotypes can also occur in time as well as in space, as in the case of the European Corn Borer (*Ostrinia nubilalis* Hübner), in which moths feeding on different host plants emerge at different times (Bethenod et al. 2005, Malausa et al. 2005), or in the case of *Enchenopa* treehoppers, in which eggs hatch at different times on different host plants and therefore have asynchronous life histories and temporal mating windows (Wood and Keese 1990). In the latter case, cohorts of treehoppers that were experimentally synchronized in the laboratory showed no behavioral tendencies towards assortative mating by host plant of origin.

On the other hand, *active* assortative mating can occur when individuals seek out sexual partners that are genetically similar to themselves by phenotype matching (Rushton 1989, Rushton and Bons 2005). For example, behavioral male preferences have been shown to produce positive assortative mating based on genetically distinct female pheromone strains in the Cabbage Looper moth (*Trichoplusia ni*) (Zhu, Chastain, Spohn,

and Haynes 1997). In addition, active assortative mating may also be based on traits that are not necessarily genetic in origin. For example, behaviorally-mediated assortative mating based on adult size has been shown in water striders (Heteroptera: Gerridae) (Rowe and Arnqvist 1996) and in meloid beetles (*Lytta magister*) (Snead and Alcock 1985). Furthermore, active assortative mating may be based upon acquired characteristics that are host-specific and based upon feeding experience. For example, in the case of the cactophilic fruit fly (*Drosophila mojavensis*), assortative mating is based on the distinct cuticular hydrocarbon compositions acquired as adults by larvae raised on different hosts, producing behaviorally-mediated reproductive isolation (Etges 1992, Brazner and Etges 1993, Etges and Ahrens 2001). The development of host-specific cuticular hydrocarbons has also been documented in the case of the bethylid wasp (*Cephalonomia tarsalis* Ashmead) (Howard 1998). Thus, assortatively mating individuals are sometimes able to discriminate potential sexual partners based on host-specific chemosensory cues.

In the case of parasitoid feeding traditions, we need to pay close attention to the proximate behavioral mechanisms presumably involved. Because of the observed plasticity in foraging behavior, the putative mechanism or behavioral predisposition for assortative mating are not likely to be under direct genetic control because there might be no simple correspondence between an individual's genotype and its host-feeding phenotype. For the same reason, philopatric patterns of dispersal (Shields 1982), the tendency to remain in the natal area through adulthood, might be similarly ineffective mechanisms for assortative mating. The major candidate for a proximate behavioral mechanism therefore appears to be phenotype matching, as has been documented in the monarch butterfly (Norton-Griffiths 1968).

There are several theoretical considerations to be taken into account when proposing assortative mating hypotheses for the Jewel wasp. There are ecological factors which might mitigate against assortative mating for host specificity. For example, the population densities of different species of hosts may vary significantly and behaviorally-inflexible daughters might then suffer, and the foraging opportunity costs of not finding a preferred host might exceed the benefits of increased offspring fecundity associated with ovipositing on the preferred host. For the Jewel wasp, it has been found that adult foraging experience may modify the behavioral effects of juvenile feeding experience to track the relative local abundance of alternative host species (Figueredo 1987, 1989). Thus, it was concluded that sympatric speciation based on host-specific assortative mating was an unlikely result of such a lifelong learning mechanism.

On the other hand, the influence of juvenile feeding experience on adult mate choice, *per se*, was not determined. Because adult experience might modify foraging behavior without altering any sexual preferences im-

printed earlier, reproductive isolation by differential juvenile feeding experience — affecting mate choice directly, rather than indirectly through diet — was still technically possible. It remained possible that Jewel wasps might mate assortatively based on juvenile feeding experience. Alternatively, because blowfly-reared females were found to be significantly more fecund (Figueredo 1987, 1989), one might instead hypothesize a possible species-typical disposition for mate choice to favor males that were blowfly-reared to maintain that host predisposition. Finally, there is also the possibility of assortative mating for other phenotypic characteristics reflecting genetic differences that are unrelated to feeding experience.

This paper reports the results of an experiment which was designed to test the effects of juvenile feeding experience on adult mate choice in the Jewel wasp. The principal hypothesis was that adult female Jewel wasps, when given a choice of males as sires for their offspring, would assortatively mate with males reared on the same juvenile host as themselves. This hypothesis was tested in the context of an alternative hypothesis that adult female Jewel wasps would assortatively mate, given the same choice conditions, on the basis of an unrelated and purely genetically determined phenotypic trait, such as eye color. The latter finding would instead support assortative mating based on genetic similarity (e.g., Rushton 1989, Rushton and Bons 2005).

METHODS

Subjects

A total of 85 adult female and 170 adult male Jewel wasps (*Nasonia vitripennis*) were used in this mate choice experiment. To accurately determine female choice of male wasps as sires for their offspring, genetically marked male and female wasps were used. Two recessive mutant genotypes were used, one for white eyes and one for red eyes (technically called “oyster” eyes and “scarlet” eyes), which, when crossed, produce hybrids with brown eyes (called the “wild” phenotype). Female wasps of both eye-color genotypes were randomly assigned to oviposit on pupae of either the carrion blowfly (*Sarcophaga bullata*) or the common housefly (*Musca domestica*). These initial ovipositing exposures were carried out in 32x102 mm plastic culture vials stoppered with foam plugs. All host fly pupae used were three to five days old as pupae and were used directly after one day of preconditioning in the incubator at LD 12:12, 25C +/- 2C, and 80%RH +/- 10%RH. Both the male and female offspring of the originally ovipositing female Jewel wasps were used as experimental subjects for this study. Cultures of all three species, including both mutant eye-color genotypes of Jewel wasp, were originally obtained from Carolina Biological Supply Company.

The experimental offspring were reared to maturity in the incubator at the photoperiods, temperatures, and humidities specified for host fly pupae. To obtain virgin females for this study, male and female subjects had to be separated from each other while they were still within the pupal stage. This was necessary because the males typically eclose a day or two earlier than their sisters, and are known to sometimes successfully inseminate female pupae. Separation of the wasp pupae was performed by carefully dissecting the pupal cases of the host flies that they had developed in and then extracting and sexing the individual wasp pupae under a microscope. This was a very delicate procedure which sometimes damaged the soft bodies of the immature wasp subjects. Moreover, housefly pupae are generally much smaller than blowfly pupae, and female Jewel wasps reared on housefly hosts are also generally smaller than those reared on blowfly hosts. The necessary handling therefore caused a certain amount of differential mortality between subjects reared on different juvenile host species. After eclosion, all adult wasps were separately housed in 15x85 mm borosilicate glass tubes stoppered with cotton plugs and fed on honey until the controlled mating and ovipositing exposures to eliminate any effects of prior feeding experience on pupae other than the larval rearing host.

PROCEDURE

All mating exposures were conducted for a standard period of 24 hours in separate 15x85 mm borosilicate glass tubes stoppered with cotton plugs under the controlled environmental conditions specified above. Adult male and female wasps were refrigerated at 6C for about 6 minutes each to facilitate handling without chemical anaesthesia and then transferred into these tubes by camel's hair brush. After the mating exposures, separate ovipositing exposures were conducted for another standard period of 24 hours in separate 32x102 mm plastic culture vials stoppered with foam plugs under the same controlled environmental conditions. Each adult female wasp was separately transferred by camel's hair brush into an individual culture vial, each of which contained one blowfly pupa for oviposition. All offspring of the experimental females were reared to maturity in these vials and then counted and classified as described below.

Experimental design

Within each test tube, every adult female was given a choice of two adult males for mating, one of each eye-color genotype, which were counterbalanced for the particular host species, either blowfly or housefly, that they had been reared on as larvae. The basic idea was that one of these males would be blowfly-reared and that the other would be housefly-reared, but that their eventual offspring could only be distinguished from each other by the particular eye-color genotype that was randomly

associated with each individual sire. Because the different mutant eye-color genotypes used might themselves influence female mate choice, however, the experiment was ultimately designed to test the relative influence of two separate factors on assortative mating in the Jewel wasp: (1) inherited eye-color genotype, and (2) conditioned foraging phenotype. Each adult male wasp in this study therefore had both an eye-color genotype and an independently assigned rearing-host phenotype. Females of independently crossed eye-color genotype and juvenile feeding experience were also used.

Data encoding

The critical information in this study was provided by the paternity of the female offspring produced by the experimental matings, but had to be statistically controlled for various related and causally prior reproductive outcomes. Because these outcomes represented the effects of various interdependent reproductive decisions made by the ovipositing females, they were not amenable to experimental control. Therefore, several dependent variables were recorded for hierarchical statistical analysis.

Because female Jewel wasps reared on housefly hosts are significantly less fertile than those reared on blowfly hosts (Figueredo 1987), we could expect significantly different total numbers of offspring (TOT), regardless of paternity, from subjects reared on different juvenile hosts. Second, a variable number of Jewel wasp offspring remain immature in a dormant condition, called diapause, for up to six months past the normal fourteen days (and, thus, the end of our experiment). The total number of such dormant immature offspring (TIO) is known to vary with certain environmental factors, such as ambient temperatures and photoperiods, but might also be affected in some unknown way by the local mating conditions experienced by the mother. Because these dormant larvae had not yet developed eyes of either color, it was not possible to determine their paternity without costly genetic analysis, perhaps producing nonrandomly missing data. Third, male Jewel wasps are always produced asexually by the mother (by parthenogenesis, or "virgin birth") and, therefore, have no biological father at all. Thus, a female Jewel wasp that does not mate with either of the males provided might produce a clutch of all male offspring. The total number of male offspring (TMO) could not be simply ignored in this study because it might have indicated female rejection of both males, and perhaps constituted more nonrandomly missing data. These first three dependent variables, although of lesser theoretical interest, were modeled hierarchically as causally prior to the last two to be able to statistically control any effect that they might have on the remaining two dependent variables.

The two dependent variables of principal interest were the total number of female offspring sired by red-

eyed, rather than white-eyed, males (TRFO), and the total number of female offspring sired by blowfly-reared, rather than housefly-reared, males (TBFO). Of course, the specified eye colors and juvenile hosts referred to are those of the fathers, rather than those of the daughters, because all genetic hybrids had brown eyes, and all posttest offspring were reared on the larger blowfly hosts for convenience (given the greater number of offspring typically produced therein). Because paternal genotypes and juvenile hosts were also slightly correlated, by the postoperative mortality described above, eye color was modeled hierarchically as causally prior to juvenile host in these two dependent variables (TRFO and TBFO).

For the same reasons, eye color was modeled hierarchically as causally prior to juvenile host in the two corresponding independent variables (FG and FJ). Consistently in direction with TRFO and TBFO, female eye-color genotype (FG) was coded 1 for red-eyed females and 0 for white-eyed females; female juvenile rearing host (FJ) was coded 1 for blowfly-reared females and 0 for housefly-reared females.

Statistical Analyses

Although the experiment seemed superficially quite simple, there were several nontrivial complications. Due to the differential mortality by rearing host alluded to above, we wound up with somewhat more blowfly-reared than housefly-reared female subjects. This also produced a slight, though nonsignificant, negative correlation between the independent variables, female genotype (FG) and female juvenile host (FJ), due to the random subject mortality across the two eye colors. Such correlated independent variables are easily modeled by hierarchical regression. To be conservative in our testing of the effects of female host, however, we assigned causal priority to female genotype. Moreover, we did not hypothesize an interaction between these two factors.

The multiple dependent variables, however, presented problems that could not be handled by unaided Multiple Regression/Correlation (MRC) techniques (Cohen and Cohen 1983). Sequential canonical analysis (SEQCA) was selected as the optimal analytical model for the present data because it partitions the covariance among multiple dependent variables sequentially, as a hierarchical regression does, while maintaining their separate identity (Gorsuch 1991, Gorsuch and Figueredo 1991). This method isolates the direct effects of the independent variables, or interventions, sequentially on each of the dependent variables or outcomes, controlling for all indirect effects through the prior dependent variables or outcomes. The only theoretical guidance required is a tentative specification of the causal order between the dependent variables (Figueredo and Gorsuch 2007). Because the two model predictor variables were binary in form, this SEQCA can also be conceptualized as mathematically equivalent to a “Step-Down” (*i.e.*,

hierarchical) Multivariate Analysis of Variance (MANOVA). This statistical method has been used previously in the study of oviposition in parasitoid wasps (Henneman et al. 1995). A full theoretical consideration of the merits and limitations of this statistical method in comparison with related methods is provided in Figueredo and Gorsuch (2007).

RESULTS

Descriptive Statistics

A total of 1072 offspring were produced by the experimental wasps. Of these, 15 were diapausing larvae at the end of the experiment and 313 were adult males. Both of these offspring categories were of indeterminate paternity and the diapausing larvae were also of undetermined sex. Of the remaining 744 adult female offspring, 256 were sired by red-eyed males and 488 were sired by white-eyed males.

Inferential Statistics

Sequential canonical analysis was performed using UNIMULT (Gorsuch 1991). All that was required for running this multivariate model was the specification of two hierarchies of causal priority, one for the two independent variables and another for the five dependent variables. The theoretical justifications for these hierarchies of causal priority were detailed above. The Pillai-Bartlett “V” Statistic (PBV) for the whole model was .21, $F(10,158) = 1.86$ ($p = .05$), which provided an overall protected test of statistical significance. Table 1 displays the overall tests of the proportions of the variance of each of the five dependent variables accounted for by the linear combinations of the two independent variables.

The SEQCA or “Step-Down” MANOVA also provided hierarchical significance tests for the separate effects of each of the two independent variables on each of the five dependent variables, as shown in Table 2.

Table 1. Multiple Correlations and Associated Tests of Significance for Each Dependent Variable in the Multivariate Model.

Variable	Effect Size	PBV	$F(2,158)$	p
TOT	R = .25	0.06	2.68	0.07
TIO	R = .14	0.03	1.31	0.3
TMO	R = .08	0.02	0.65	n.s.
TRFO	R = .32	0.15	7.06	< 0.005
TBFO	R = .12	0.02	0.73	n.s.

Table 2. Semipartial Correlations and Associated Tests of Significance for Each Independent Variable in the Multivariate Model.

Variable	Effect Size	PBV	F(1,158)	p
Dependent variable: TOT				
FG	r = -.13	0.02	1.43	0.2
FJ	r = .22	0.05	3.93	0.05
Dependent variable: TIO				
FG	r = -.14	0.03	2.62	0.1
FJ	r = .00	0	0	n.s.
Dependent variable: TMO				
FG	r = -.08	0.02	1.25	0.3
FJ	r = .02	0	0.05	n.s.
Dependent variable: TRFO				
FG	r = .31	0.14	13	<.001
FJ	r = -.09	0.01	1.12	0.3
Dependent variable: TBFO				
FG	r = .12	0.02	1.44	0.2
FJ	r = .01	0	0.02	n.s.

The standardized regression coefficients (“beta-weights”) obtained by SEQCA are shown in Table 3.

Thus, controlling for all the prior dependent variables, there were only two statistically significant effects of the independent variables on the dependent variables. These significant effects were: (1) the predicted effect of female juvenile rearing host (FJ) on total number of offspring (TOT), and (2) the effect of female genotype (FG) on total number of female offspring sired by red-eyed males (TRFO).

These findings indicate that: (1) blowfly-reared experimental females produced more offspring in general than did housefly-reared females (beta=.217), and (2) red-eyed experimental females produced more female offspring sired by red-eyed males than did white-eyed females (beta=.300). The former is a previously documented effect of rearing host (Figueredo 1987, 1989) and the latter constitutes evidence for positive assortative mating for eye color. The mean number of offspring in all categories combined produced by blowfly-reared females was 14.52 and that produced by housefly-reared females was only 6.81, both regardless of eye-color genotype. The mean number of female offspring sired by red-eyed males produced by red-eyed

Table 3. Standardized Regression Coefficients for Each Independent Variable in the Multivariate Model.

TOT	=	-.105*FG	+ .217*FJ
TIO	=	-.144*FG	+ .004*FJ
TMO	=	-.080*FG	+ .016*FJ
TRFO	=	.300*FG	- .092*FJ
TBFO	=	.121*FG	+ .012*FJ

females was 4.46 and that produced by white-eyed females was only 1.14, both regardless of juvenile rearing host.

When these means are statistically adjusted for the effects of all prior dependent variables, they are 4.90 and 1.36, respectively, which represent an even larger difference.

The most important negative result of this analysis is that female juvenile rearing host (FJ) had no significant effect on the total number of female offspring sired by blowfly-reared males (TBFO). This latter result represents empirical evidence against the hypothesis that there is positive assortative mating for juvenile rearing host in the Jewel wasp. When these means are statistically adjusted for the effects of all prior dependent variables, they are 4.09 and 4.15, respectively, which would hardly indicate a difference of any consequence even had it reached statistical significance.

DISCUSSION

Our study revealed no evidence of assortative mating by juvenile feeding experience in the Jewel wasp. It is therefore unlikely that differential juvenile feeding experience can lead to sympatric speciation in this particular species by either of the mechanisms that have been proposed. The requisite reproductive isolation can apparently not be produced by differential juvenile feeding experience either indirectly by permanently differentiated foraging behavior or directly by differentially imprinted sexual preferences. Instead, assortative mating was shown to be greatly influenced by genetic markers, such as eye color.

Like many other species, the Jewel wasp does assortatively mate. Although the mutant eye-color genotypes used in this experiment were artificially selected laboratory strains, assortment by eye color was probably indicative of a preference for the greater set of genetic similarities that presumably existed between the members of the same recessive mutant strains. The selective breeding of these two recessive mutant strains was likely to have resulted in homozygosity on a variety of other

traits as well as on eye color. It is therefore possible that eye color, *per se*, was not even directly perceived or used as markers by the wasps as proximate cue for mate discrimination, but that the relevant genetic information regarding relatedness was carried instead by other indicators of phenotypic similarity, such as olfactory cues. Assortative mating is thought to function in the wild to maintain specific homozygous genotypic adaptations and reduce the costs of mating (Bateson 1983). Assortative mating tracks local selective forces and thus allows local populations to become genetically better adapted to local circumstances in which surviving genotypes have been successful (see also Kawecki 1994 for other genetic benefits). Why, then, do Jewel wasps not assortatively mate by host-feeding experience? Should not similar benefits accrue?

The advantage of specialization to a single host species is that the parasitoid can maximize its adaptations to that particular host's physiology and lifecycle and thus reduce its competition with generalists. The disadvantage is that complete dependence on a single fluctuating host population may indirectly result in the occasional extinction of local parasitoid populations. The most likely reason for the absence of either natural or sexual selection for more restricted host specificity in the Jewel wasp might therefore reside in the nature of the host fly life histories. Because the host flies feed on ephemeral resources, such as animal carcasses, fly populations are locally unstable. As colonizers, the first pioneering fly species to find a favorable microenvironment temporarily becomes the locally predominant species. When these ephemeral resources are exhausted, however, the local population of that host species then precipitously crashes. Proverbially, they breed like flies and they die like flies (Figueredo 1987, 1989). Any parasitoid specializing on such a host species will experience large shifts in host abundance. If there were any genetic variance in the restrictedness of host selection by the parasitoid, this fluctuating food supply could result in the elimination of any more restricted feeding genotypes and the survival of any less restricted feeding genotypes which were able to utilize any of a variety of fly species that colonized the local area.

As demonstrated in adult foraging behavior (Figueredo 1987, 1989), Jewel wasps display a lifelong conditional feeding strategy based on the relative abundances of alternative host species. The host species on which an individual wasp was reared influences but does not permanently limit its foraging options as an adult. A more restricted host preference, whether environmental or genetic in origin, would force the wasp to search until a host of a specific type is found, even in cases where acceptable substitutes were available in the immediate vicinity. Assortative mating for host-feeding phenotype would therefore impose an additional foraging cost on the offspring that is evidently suboptimal in the adult. In the absence of lifelong host fidelity in the adult (which

could lead to some microgeographic segregation and, thus, to passive assortative mating), the most plausible mechanism remaining for sympatric speciation was thus active assortative mating for prior host-feeding experience. This hypothesis was disconfirmed. Although Jewel wasp females were found to be mating assortatively based on manipulated markers of genetic relatedness (presumably indicated by phenotypic similarity), they were not found to be mating assortatively based on host-feeding phenotype. We can only conclude that both of the plausible mechanisms proposed for reproductive isolation leading to sympatric speciation, passive assortative mating by permanently differentiated foraging behavior and active assortative mating by differentially imprinted sexual preferences, are absent in the Jewel wasp. Behaviorally flexible parasitoid species like the Jewel wasp are thus likely to remain polyphagous.

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