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Differences in mating behaviour and sex ratio between three sibling species of *Nasonia*

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ABSTRACT

Mating in *Nasonia* wasps has traditionally been thought to occur on or around their pupal fly host, after emergence. Here we report the occurrence of within-host mating (WHM) in *Nasonia*. Within-host mating is interesting as a simple behavioural trait that can have a strong effect both on the level of inbreeding and the level of interspecific mating in a species. A survey of WHM levels was performed on 17 recently collected strains of *Nasonia* from three sibling species: *N. vitripennis*, *N. giraulti* and *N. longicornis*. Both *N. giraulti* and *N. longicornis* mated within hosts at significantly higher rates than *N. vitripennis*. The mean (±S.D.) percentage of females mating within hosts was 64.4 ± 16.6 in *N. giraulti*, 9.1 ± 8.5 in *N. longicornis* and 1.0 ± 2.1 in *N. vitripennis*. Within-host mating in *N. giraulti* and *N. longicornis* may represent a mechanism for escaping hybridization with *N. vitripennis*, which often co-occurs microsympatrically in birds' nests with the other two allopatric species. Since WHM presumably increases local mate competition (LMC) and inbreeding among progeny, elevated levels of WHM should select for more female-biased sex ratios. Mean one-foundress sex ratios were calculated for 20 *Nasonia* strains, with most of these strains being the same as those in the general WHM assay. As expected, *N. giraulti* had significantly more female-biased sex ratios than both *N. longicornis* and *N. vitripennis*. The implications of WHM for the population dynamics of *Nasonia* are discussed.

Keywords: adaptation, inbreeding, local mate competition, mating behaviour, *Nasonia*, sex ratio, sibling species.

INTRODUCTION

Behaviour is generally believed to be a trait that can evolve quickly in diverging populations and species. Thus, closely related species can often differ behaviourally from each other. Such behavioural differences can, in turn, have cascading effects on other aspects of biology, including morphology, physiology and genetics. Here we report a simple behavioural difference between three closely related species of *Nasonia* wasps that has profound effects on population structure, sex ratio selection and the potential for interspecific hybridization.

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N. vitripennis and its two recently discovered sibling species, *N. giraulti* and *N. longicornis*, are small (2–3 mm) wasps that parasitize various species of fly pupae (Darling and Werren, 1990). *N. giraulti* and *N. longicornis* specialize on the pupae of various blowfly species commonly found in birds' nests (Darling and Werren, 1990), while *N. vitripennis* is a host generalist, parasitizing a wide range of flies in a variety of locations, including birds' nests and animal carcasses (Whiting, 1967; Werren, 1983). *N. giraulti* and *N. longicornis* occur sympatrically with the cosmopolitan *N. vitripennis* in the northeastern and western USA, respectively, and often occur microsympatrically with *N. vitripennis* in individual birds' nests (Darling and Werren, 1990). *N. giraulti* and *N. longicornis* are allopatric. *Nasonia*, like most wasps, have haplodiploid sex determination (Cook, 1993; Beukeboom, 1995; Cook and Crozier, 1995).

Although few studies have been conducted on the sex ratios of *N. giraulti* and *N. longicornis* (but see King and Skinner, 1991), female-biased sex ratios are well documented in *N. vitripennis* (Velthuis *et al.*, 1965; Wylie, 1966; Walker, 1967; Werren, 1980, 1983, 1984; Orzack, 1986). In this species, males have vestigial wings and are incapable of flying, which leads to variable levels of local mate competition and inbreeding, explaining the observed sex ratio bias (Hamilton, 1967; Charnov, 1982; Werren, 1983; Werren and Simbolotti, 1989; Molbo and Parker, 1996). The possibility exists that *N. longicornis* and/or *N. giraulti* males may disperse after emergence, as their wings are larger than those of *N. vitripennis* males, and there is some evidence that the males of these two species can fly (King and Skinner, 1991; J. Werren, unpublished data). Dispersal from the natal patch should lower local mate competition and inbreeding and select for less female-biased sex ratios (Nunney and Luck, 1988; Werren and Simbolotti, 1989). Male flight and dispersal in *Nasonia* have not been investigated thoroughly. In *Nasonia* wasps and many other parasitoids, the sex of progeny is under the behavioural control of the mother. Because of this, it is possible for natural selection to produce adapted sex ratios (Werren, 1980, 1983; Charnov, 1982; but see Orzack, 1986; Orzack and Parker, 1986).

Although mating in *Nasonia* has traditionally been thought to take place on or around the host pupae after emergence (Whiting, 1967), laboratory observations have indicated that wasps from a *N. giraulti* strain (RV2) mated within their host before emerging. Within-host mating (WHM) is rare in parasitoids, and has been documented in only a few species (da Costa Lima, 1928; Suzuki and Hiehata, 1985). The occurrence of WHM in natural populations of *Nasonia* would have large effects on population structure and inbreeding levels, and therefore on the optimal sex ratio (Hamilton, 1967). In the most extreme case, 100% WHM occurring in a host parasitized by a single foundress would result in complete sibmating. In such a context, competition among brothers for mates increases, and genetic relatedness of females to daughters and sons increases, but relatively more to daughters (Suzuki and Iwasa, 1980; Frank, 1985; Herre, 1985; Werren, 1987). Both of these factors are expected to select for even more female-biased sex ratios in populations of *Nasonia* which mate within their hosts. The amount of WHM should determine the magnitude of the deviation from the expected sex ratio.

Within-host mating also has potential implications for interspecies hybridization. *Nasonia* species are microsympatric, often occurring in the same bird nests (Darling and Werren, 1990). Laboratory studies have indicated that interspecies mating can readily occur. However, WHM may reduce the frequency of interspecies hybridization, and could be a mechanism for escape from hybridization. Therefore, it is important to determine the frequency of WHM in the three species.

In this study, experiments were performed to (1) characterize the WHM behaviour in strains of three *Nasonia* species, (2) characterize the sex ratio behaviour of the three species, and (3) determine if sex ratio varies with the frequency of WHM at the species level, as predicted by sex ratio theory.

MATERIALS AND METHODS

Stocks

Stock cultures of wasps were raised in constant light at 25°C. Wasps are normally placed in vials *en masse* and presented with 15–20 *Sarcophaga bullata* hosts to parasitize. The strains used in this study were: *N. vitripennis* strains NVNY D554, NVOH 204, NVEUS 3, NVEUS 5, NVPa 213E, NVNY D553, NVNY D555, NVUTN 350C and NVXIDB 430AM; *N. giraulti* strains NGVa 203Bi, NGV 201AR, NGPa 233F2, RNGPa 233F2, RNGOH 206D and NGOH 206D; and *N. longicornis* strains NLUT 218, NLCA 9304, NLNV 206H, NLUT 220B, NLUT 214, NLIDB 418A and NLUTN 350C. Most of these strains were used in both the WHM and sex ratio assays. They were collected recently (within 1–5 years) and then maintained primarily in diapause. During culturing under short photoperiod and cool conditions, *Nasonia* larvae may enter a diapause, or hibernation, and can then be maintained for up to 2 years at a time by storing them at cold temperatures (~4°C). These diapaused lines therefore experienced few generations since being collected from nature.

In addition, WHM rates were determined in the *N. giraulti* strain RV2 and three strains derived from RV2 [RV2T, RV2T(old) and RV2R]. RV2 was established from a single mated female collected from Virginia in 1987. It has been maintained primarily by active culturing at 25°C (2 week generation time) since. In 1987, RV2T was derived from RV2 by tetracycline treatment to cure the strain of the endosymbiont *Wolbachia* (Breeuwer and Werren, 1990). This line has also been maintained primarily in laboratory culture since. RV2T(old) is an RV2T subline that was placed in diapause around April 1995 and removed around June 1996, a time during which RV2T had been maintained in active culture. Therefore, it could be used for comparisons of WHM rate. Finally, RV2R was derived from RV2T by rifampicin treatment to cure it of *Wolbachia* in May 1996. Thus, each of these lines has a different history, although derived from a common ancestor.

Testing strains for mean WHM levels

Within-host mating rates were measured in nine *N. vitripennis*, four *N. giraulti* and four *N. longicornis* strains. One generation prior to testing, mated females were taken from stock cultures and provided with hosts in groups of five females and five hosts (a total of 80 hosts per strain). After allowing the females 4 days to parasitize the hosts, the wasps were removed and the hosts were separated into individual vials. Approximately 14 days after initial presentation of the hosts to the females, adult wasps began to emerge from the hosts. When this was observed in 15–30% of the vials, hosts from the remaining vials were opened individually and the contents were separated on a white foam surface. At this stage, wasps are typically eclosed adults that have not yet emerged from the host. After the presence of eclosed males was noted, all males and any females near them were destroyed. A number of the remaining eclosed females were individually isolated in glass vials. Isolation of eclosed adults from a host was typically completed within 1 min of opening the host. Thus, females were collected before they could mate outside the host. Hosts were used for female collections only if at least 50% of the wasps were eclosed. After 24 h in isolation, each collected female was given two *S. bullata* hosts to parasitize. The sample sizes (number of females tested) were as follows:

- *N. vitripennis* strains: NVOH 204 (134), NVEUS 3 (85), NVEUS 5 (97), NVPa 213E (124), NVNY D553 (118), NVNY D554 (123), NVNY D555 (127), NVXIDB 430AM (108) and NVUTN 350C (91).
- *N. giraulti* strains: NGVa 203Bi (79), NGV 201AR (109), NGPa 233F2 (69) and RNGOH 206D (82).
- *N. longicornis* strains: NLUT 218 (91), NLCA 9304 (136), NLIDB 418A (71) and NLUTN 350C (97).

The F_1 progeny of these females were scored according to sex. Because *Nasonia* are haplodiploid and non-thelytokus, if a brood was a mixture of males and females, then the mother had mated; if the brood consisted of all males, then the mother was a virgin.

Control for possible thelytoky

Although *Nasonia* are not known to produce female progeny by female parthenogenesis (thelytoky), a control for daughter production by unmated females was performed. This control was carried out using *N. giraulti* strains NGOH 206D and NGPa 233F2, which had both exhibited relatively high WHM rates in previous tests. A test for WHM was performed as described before. However, instead of isolating unemerged adult females from hosts, some females were isolated as pupae (which are obviously unable to mate within-the-host). Females of both groups were given two *S. bullata* hosts at the same time and F_1 progeny were scored as previously stated. No females isolated as pupae produced female progeny ($n = 58$, NGOH 206D; $n = 57$, NGPa 233F2), in comparison to those isolated as eclosed adults ($n = 61$ and $n = 73$, respectively) ($P < 0.001$, d.f. = 1, χ^2). This result demonstrates that, as expected, *Nasonia* wasps do not exhibit thelytoky.

Outside-the-host mating control

It is possible that a female could mate in the brief time between when a host is opened and when the female is collected, although great care was exercised to exclude this possibility. To determine whether this occurred, we ran a separate test using *N. giraulti* strain RNGPa 233F2, which exhibited a relatively high frequency of WHM in previous tests. For a control, a test for WHM was performed as described above with approximately half the hosts. The contents of the other half were placed onto a CO_2 plate, which anaesthetized the wasps and therefore eliminated the possibility of outside-the-host mating. Females generally regained consciousness within 1 min of being isolated in a vial. Females of both groups were given two *S. bullata* hosts at the same time and F_1 progeny were scored as previously described. We found no significant differences in WHM frequency between females isolated with CO_2 and those isolated without ($0.2 < P < 0.3$, d.f. = 1, χ^2), indicating that the amount of error in these assays due to the occurrence of outside-the-host mating was low to none.

Measuring sex ratios

Mated females from stock cultures were isolated singly in 12 × 75 mm glass vials and given two *S. bullata* hosts each. They were allowed to parasitize these until death (typically after 5 days at 25°C). All females were 3–4 days old at the time of isolation and hosting. F_1 progeny were allowed to emerge and die, and were scored according to sex. Sex ratios were calculated as the proportion of males in each brood, excluding any diapaused larvae. Sex ratios were not assayed in multiple-foundress instances (e.g. four females on four hosts) because of the differential attractiveness of *S. bullata* hosts to the two species. *N. giraulti* and *N. longicornis* females prefer *Protocalliphora* to *S. bullata* hosts, and their stinging rates on *S. bullata* are often about 50%, compared to near 100% for *N. vitripennis*. Therefore, the actual number of stinging females in a four-foundress context could be quite different for the two species, thus giving spurious results for sex ratios. It is for this reason that we tested sex ratios in only the single-foundress context.

Changes in WHM frequency during laboratory culturing

Preliminary results suggested that sublines of a *N. giraulti* strain (RV2) that had been maintained under laboratory culturing for several years had changed in WHM rate. Within-host mating frequencies were determined for RV2 and its sublines [RV2T, RV2T(old) and RV2R; all derived from strain RV2 by antibiotic curing of the endosymbiont *Wolbachia* (Breeuwer and Werren, 1990)]. Changes in WHM rate were documented by assaying frequencies of WHM through time, and by comparing strains kept in diapause versus by continuous culturing.

RESULTS

WHM rates in the three *Nasonia* species

Within-host mating levels for nine *N. vitripennis*, four *N. giraulti* and four *N. longicornis* strains were determined (Fig. 1). Each of these strains was established from single mated females collected in the field, and had been maintained primarily under diapause. Therefore, the strains had experienced only a few generations under laboratory culturing since their collection. The three species clearly differed in overall WHM frequencies ($P < 0.01$, d.f. = 2, Kruskall-Wallis). Both *N. giraulti* and *N. longicornis* had significantly higher WHM rates than *N. vitripennis* ($P = 0.0055$ and $P = 0.0214$, respectively, MWU with a Bonferonni correction for multiple comparisons). Using each strain as a datum, the mean (±S.D.) percentage of females mating within hosts was 64.4 ± 16.6 in *N. giraulti* ($n = 4$), 9.1 ± 8.5 in *N. longicornis* ($n = 4$) and 1.0 ± 2.1 in *N. vitripennis* ($n = 9$).

Sex ratio differences

To investigate sex ratio differences between the species, single females were presented with two hosts to parasitize, and the progeny sex ratio was scored. Results of the one-foundress sex ratio test are shown in Table 1. Using mean sex ratios calculated for each strain, overall significant differences were found between the strains ($P < 0.01$, d.f. = 2, Kruskall-Wallis). In direct species comparisons, *N. giraulti* was found to have significantly lower sex ratios (proportion males) than both of the other two species ($P < 0.01$, MWU, with a Bonferonni

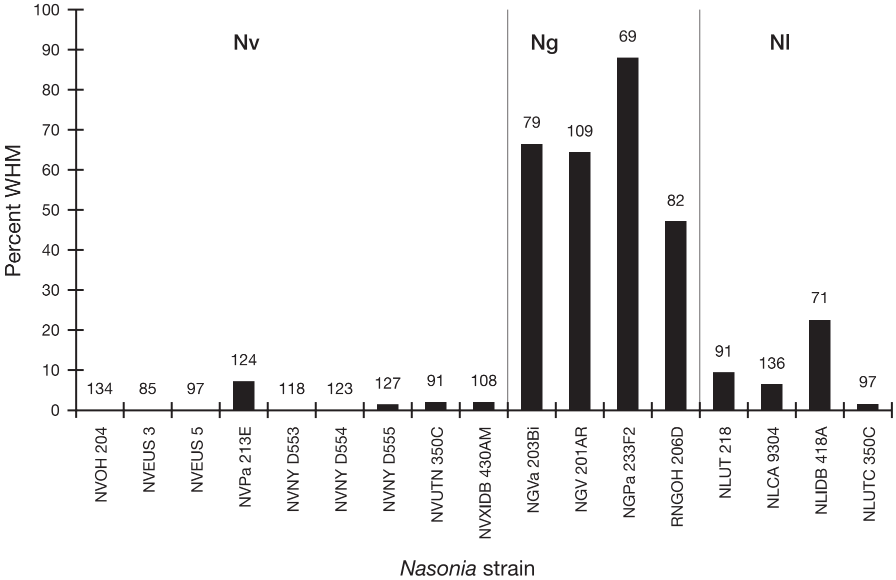

Fig. 1. Levels of within-host mating in three species of *Nasonia*. The WHM rates of *N. giraulti* (Ng) and *N. longicornis* (Nl) are significantly greater than those of *N. vitripennis* (Nv). See the text for details on strains and methods used. Sample sizes (number of females tested) are shown above bars.

Table 1. Results of *Nasonia* sex ratio assay (mean ± s.d.)[a]

Species	Strain	Males	Females	Brood	Sex ratio	*n*
N. vitripennis	NVOH 204	12.7 ± 4.2	80.9 ± 23.1	93.6 ± 22.7	0.15 ± 0.08	10
	NVEUS 5	13.2 ± 2.9	86.9 ± 17.0	100.1 ± 19.0	0.13 ± 0.02	13
	NVPa 213E	7.6 ± 3.6	53.7 ± 14.0	61.3 ± 15.5	0.12 ± 0.05	19
	NVNY D553	13.5 ± 6.9	84.8 ± 21.1	98.3 ± 21.8	0.14 ± 0.06	25
	NVNY D554	13.0 ± 7.0	70.1 ± 26.0	83.1 ± 30.7	0.16 ± 0.05	8
	NVNY D555	12.8 ± 12.1	79.4 ± 21.3	92.2 ± 22.1	0.14 ± 0.10	27
	NVUTN 350C	11.0 ± 8.8	53.2 ± 20.7	64.2 ± 25.0	0.17 ± 0.09	14
	NVXIDB 430AM	11.0 ± 5.2	68.3 ± 26.5	79.3 ± 30.5	0.14 ± 0.04	16
N. giraulti	NGVa 203Bi	6.5 ± 3.5	87.6 ± 21.3	94.1 ± 22.7	0.07 ± 0.03	28
	NGV 201AR	5.4 ± 2.1	99.8 ± 23.4	105.2 ± 24.1	0.05 ± 0.02	26
	NGPa 233F2	6.8 ± 2.4	88.4 ± 27.1	95.2 ± 28.5	0.08 ± 0.02	18
	NGOH 206D	6.3 ± 2.1	97.6 ± 13.6	103.9 ± 14.9	0.06 ± 0.02	9
	RNGOH 206D	7.4 ± 3.5	95.8 ± 18.0	103.2 ± 18.2	0.07 ± 0.04	16
N. longicornis	NLUT 218	6.6 ± 3.7	69.8 ± 17.6	76.4 ± 19.1	0.09 ± 0.04	25
	NLCA 9304	10.7 ± 7.3	80.7 ± 18.9	91.4 ± 17.8	0.12 ± 0.09	12
	NLIDB 418A	5.3 ± 2.5	41.3 ± 20.0	46.6 ± 19.4	0.11 ± 0.10	3
	NLUTC 350C	9.7 ± 3.0	64.8 ± 24.0	74.5 ± 25.3	0.13 ± 0.05	16
	NLNV 206H	10.1 ± 4.6	58.5 ± 18.7	68.6 ± 21.9	0.15 ± 0.04	14
	NLUT 220B	7.1 ± 4.2	68.9 ± 26.1	76.0 ± 30.2	0.09 ± 0.03	7
	NLUT 214	11.6 ± 4.2	65.4 ± 17.8	77.0 ± 20.0	0.15 ± 0.04	17

[a] Single females were tested as described in the 'Materials and methods' section. *n* = the number of families.

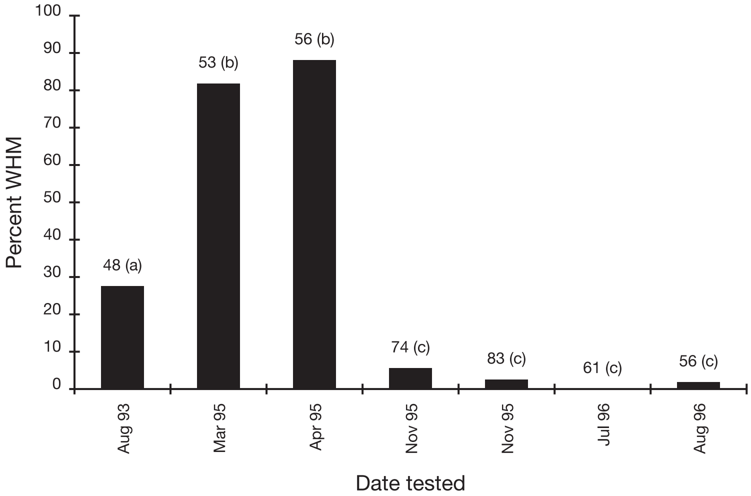

Fig. 2. Change in WHM frequency through time in the *N. giraulti* strain RV2T. The WHM levels in RV2T decreased significantly through time. The numbers above each bar represent the total number of females tested.

correction). Using each strain as a datum, the mean sex ratios for *N. giraulti*, *N. longicornis* and *N. vitripennis* were 0.06 ± 0.01 ($n = 5$), 0.12 ± 0.03 ($n = 7$) and 0.14 ± 0.02 ($n = 8$), respectively. *N. longicornis* family sizes were found to be significantly smaller than those of both *N. giraulti* and *N. vitripennis* ($P < 0.05$, MWU).

These experimental data are consistent with sex ratios found in parasitized *Protocalliphora* hosts collected from bird boxes in Geneseo, NY in June 1996 (M. Drapeau and S. Bordenstein, unpublished data). In a sample of 67 *N. vitripennis*-containing and 19 *N. giraulti*-containing hosts, *N. giraulti* was found to have a significantly lower sex ratio than *N. vitripennis* ($P < 0.001$, MWU), mainly due to a significant decrease in the number of males ($P < 0.001$, MWU).

Effect of prolonged culture on WHM rates

We found significant variation for WHM rates within a closely related group of *N. giraulti* strains. Three strains – RV2T, RV2R and RV2T(old) – were all derived from strain RV2 following antibiotic treatment. Specifically, RV2 and RV2T have very low rates of WHM [3.9% ($n = 102$ females) and 0.9% ($n = 117$), respectively], in contrast to RV2R and RV2T(old), which have relatively high rates [68.7% ($n = 83$) and 66.0% ($n = 47$), respectively]. RV2 and RV2T had previously been shown to have high WHM rates [RV2 = 92.3% ($n = 33$ females, February 1991), RV2T = 81.1% ($n = 53$ females, March 1995); G. Sinha and J. Werren, unpublished data], apparently losing the ability to mate within hosts over a relatively short amount of time. A significant decrease in WHM frequency of strain RV2T was documented in assays performed between August 1993 and August 1996 (Fig. 2). Bars labelled with the same letter (a, b or c) in Fig. 2 did not differ significantly in WHM frequency. Bars with different letters had significantly different WHM rates ($P < 0.001$, d.f. = 1, χ^2). These results demonstrate that WHM can change in frequency through time in continuously maintained cultures.

Table 2. Within-host mating frequencies in strains RV2T(old) and RV2T[a]

	July 1996		August 1996	
Strain	WHM frequency	*n* (females)	WHM frequency	*n* (females)
RV2T	0.0	61	1.8	56
RV2T(old)	66.0	47	74.5	51

[a] The strain RV2T(old) had been maintained in diapause from April 1995 to June 1996. Whereas RV2T was maintained in active culture. The WHM levels in RV2T(old) resemble those originally shown by RV2T (the 'ancestral state') (see Fig. 3).

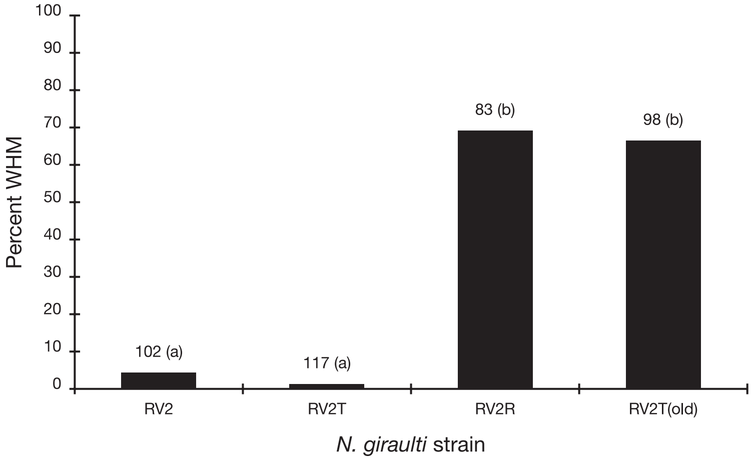

Fig. 3. Variation in WHM in the RV2 family of *N. giraulti* strains. Strains RV2T, RV2R and RV2T (old) have all been derived from strain RV2 following antibiotic treatment, yet large differences are seen in their WHM levels. The numbers above each bar represent the total number of females tested.

Specific differences in WHM rates between strains maintained in diapause versus continuous culturing were also noted. RV2T(old) had been maintained under diapause from April 1995 to June 1996, whereas RV2T was maintained in serial cultures at 25°C during this time (approximately one generation every 15 days). Table 2 shows WHM frequencies of RV2T and RV2T(old) when tested contemporaneously in both July and August 1996. Significant differences were found in both comparisons ($P < 0.01$, d.f. = 1, χ^2). This test supports the view that WHM rates changed in RV2T between April 1995 and July 1996.

In addition, comparisons of the WHM rates of the four RV2-derived strains are shown in Fig. 3. These strains were tested at the same time as the other 17 *Nasonia* strains in the general WHM assay. Strains labelled with the same letter (a or b) in Fig. 3 did not differ significantly at the $P = 0.05$ level. There are clearly significant interstrain differences in WHM rate when strains RV2R and RV2T(old) are compared to both strains RV2 and RV2T.

As seen in Table 3, WHM frequency and sex ratio appear to co-vary in the RV2-derived strains. RV2 and RV2T have both low WHM rates and relatively high sex ratios (proportion males). In comparison, RV2R has a much greater WHM frequency and a lower sex ratio,

Table 3. Within-host mating frequencies and sex ratios in RV2-derived strains[a]

Strain	WHM		Sex ratio	
	Frequency	*n* (females)	Mean ± S.D.	*n* (females)
RV2 (a)	3.9	102	0.10 ± 0.04	18
RV2T (a)	0.9	117	0.13 ± 0.06	42
RV2R (b)	68.7	83	0.05 ± 0.02	23

[a] Strains designated by different letters differ significantly from each other in both WHM frequency ($P < 0.01$, d.f. = 1, χ^2) and sex ratio, expressed as the proportion of males ($P < 0.01$, MWU).

not significantly different from those of the *N. giraulti* field lines (Fig. 1). Thus, both WHM and sex ratio appear to have changed in these commonly derived sublines.

DISCUSSION

In this study, significant differences in within-host mating frequency and sex ratio were found between three closely related *Nasonia* species. Within-host mating rate and sex ratio were also found to change through time in some laboratory cultures of *N. giraulti.* Although WHM may have deleterious effects, such as increasing the mean level of inbreeding, there are a number of possible explanations for why WHM has evolved in *N. giraulti* and *N. longicornis*. One possibility is that it may be selectively advantageous for *N. giraulti* and *N. longicornis* to mate within hosts as a mechanism for escaping hybridization with *N. vitripennis*. Because hybrids formed between the species have lower fitness than those of a cross involving members of the same species (Breeuwer and Werren, 1990, 1995; J. Werren, unpublished data), an individual wasp gains fitness if it mates conspecifically. Maladaptive hybridization will have a greater effect on the species with the smaller population size; in this case, *N. giraulti* or *N. longicornis*. *N. vitripennis* has a much greater host range (Darling and Werren, 1990) and population size (J. Werren, unpublished data). Within-host mating could therefore act as a form of premating isolation. Mating within a host will always increase the chances of mating with a member of the same species, except in the case of multiparasitism (when more than one species parasitizes the same host) (Smith, 1916; Godfray, 1994), which we estimate to be low based on field studies (Darling and Werren, 1990; M. Drapeau and S. Bordenstein, unpublished data). Although the females that mate within the host could also mate outside the host, *N. vitripennis* (and presumably *N. giraulti* and *N. longicornis*) show strong first male sperm precendence (van den Assem and Visser, 1976). If WHM has evolved as an escape mechanism from hybridization, it is likely to be an adaptive response with long-term costs. Lineages with high WHM rates will be highly inbred and may therefore accumulate deleterious mutations at a higher rate (Charlesworth *et al.*, 1990; Werren, 1993).

Another possibility is that WHM frequency may be a pleiotropic consequence of another trait. For example, female receptivity to interspecific males appears to be correlated with WHM frequency (M. Drapeau and S. Bordenstein, unpublished data), lending evidence to the pleiotropy theory. However, the pattern is that *N. giraulti* females from strains with high

WHM show greater willingness to mate interspecifically in controlled crossing experiments. Thus, an alternative explanation is that WHM selects for reduced mate discrimination and outside-the-host mating for greater mate discrimination, rather than WHM being a pleiotropic side-effect of selection for female interspecific receptivity. The genetic basis of these traits has yet to be determined.

It is interesting to note that a behaviour such as WHM that can cause rapid reproductive isolation can also cause sympatric speciation. We are not suggesting that the *Nasonia* complex evolved by sympatric speciation; in fact, our bias is that the species evolved allopatrically, and that WHM evolved after secondary sympatry as an escape from hybridization. However, we do wish to point out that WHM is the type of behaviour which could lead to rapid isolation, a necessary requirement for sympatric speciation (Mayr, 1942, 1963; King, 1993; Ridley, 1993).

A second major consequence of WHM is its effects on sex ratio selection. Various hypotheses have previously been suggested to explain the differences in sex ratio between *N. vitripennis* and *N. giraulti*, including differences in egg-to-adult mortality, maternally inherited sex ratio factors, dispersal differences and non-synchronization of hosts in nature (King and Skinner, 1991). However, the occurrence of WHM is probably sufficient to explain the sex ratio biases. Within-host mating presumably increases levels of local mate competition and inbreeding, and a more female-biased sex ratio should be selected for.

The decrease in WHM frequency in some RV2-derived laboratory cultures is interesting, and has several possible explanations. The first question is, how did the genetic variation for WHM arise in these highly inbred isofemale lines? One explanation is that variability in RV2 and RV2T arose by spontaneous mutation during the last 10 years of continuous culturing in the laboratory, which is approximately 250 generations. Another possibility is that genetic variability may have arisen through contamination of the strains by one of the other two species. This explanation would require selective introgression of the genes involved in WHM and sex ratio, because RV2 and RV2T are clearly *N. giraulti* in other respects (e.g. wing size). Preliminary data gathered using RAPD markers indicate that some *N. vitripennis* markers have indeed been introgressed into the RV2T lines, suggesting contamination (J. Gadau, personal communication). Once genetic variability for WHM was present in these cultures, WHM may have been selected against to reduce the mutational load resulting from high inbreeding levels. Individuals mating outside the host can genetically recombine with other lineages; as a result, genomes with lower numbers of deleterious mutations would be reconstituted (Crow, 1988, 1994).

Also of interest is the apparent correlated change in sex ratio allocation in the two strains with reduced WHM rates. Although this may have occurred by chance, it is interesting that the sex ratio changed in the direction which would be favoured by natural selection (i.e. higher sex ratios with lower WHM). Behavioural changes in laboratory cultures of *Nasonia* are not unprecedented; J. van den Assem (personal communication) has documented changes in courtship behaviour and female receptivity of *N. vitripennis* over a period of 40 years.

The occurrence of WHM in nature could have serious consequences for the population structure and evolution of *Nasonia* species. Although haplodiploid organisms can more readily handle extreme inbreeding (Werren, 1993), generally a high level of inbreeding will lead to an increase in the accumulation of deleterious mutations, and for this reason selection against WHM might be expected (Charlesworth *et al.*, 1990). However, the level of inbreeding caused by WHM may be decreased by superparasitism (Werren, 1980;

Herre, 1985; Molbo and Parker, 1996). One potential avenue of future research would be to estimate inbreeding levels in natural populations of *N. giraulti* and *N. longicornis* by using allozyme markers, as has been done recently with populations of *N. vitripennis* (Molbo and Parker, 1996). By doing this, natural mating structures of closely related species can begin to be quantified and compared based on inferences made in the laboratory.

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