8-2003

Temperature effects on emergence time, proportion of males, and diapause in three species of Nasonia (Hymenoptera: Pteromalidae)

Anne Grinnan

To the Graduate Council:

I am submitting herewith a thesis written by Anne Grinnan entitled "Temperature effects on emergence time, proportion of males, and diapause in three species of Nasonia (Hymenoptera: Pteromalidae)." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Christine Boake, Major Professor

We have read this thesis and recommend its acceptance:
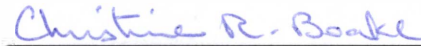
Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Anne Grinnan entitled "Temperature Effects on Emergence Time, Proportion of Males, and Diapause in Three Species of *Nasonia* (HYMENOPTERA: PTEROMALIDAE)". I have examined the final paper copy of the thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

_____
Christine Boake, Major Professor
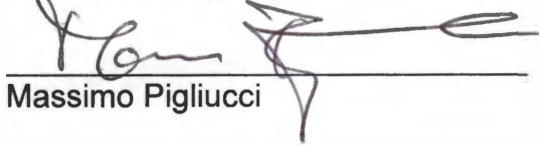
We have read this thesis
and recommend its acceptance:

_____
Susan Riechert

_____
Massimo Pigliucci

Accepted for the Council:

_____
Vice Provost and Dean of Graduate Studies

Temperature Effects on Emergence Time, Proportion of Males, and Diapause in
Three Species of *Nasonia* (HYMENOPTERA: PTEROMALIDAE)

A Thesis presented for the Master of Science Degree

The University of Tennessee, Knoxville

Anne Grinnan

August 2003

Abstract

Temperature has been demonstrated to induce phenotypic plasticity in numerous insects for numerous traits. An organism of a specific genotype is said to exhibit phenotypic plasticity if it shows variation in a trait or traits that is dependant on changes in the external environment. A reacti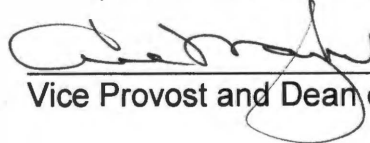on norm is the function that relates the environments to which a particular genotype is exposed and the phenotypes that can be produced by that genotype. Genotype-by-environment interactions measure how genotypes vary in their reaction norms. Trait responses to temperature can be examined with analyses that test for G by E interactions and display norms of reaction; thus telling us if different genotypes behave differently in different temperature environments.

In the genus *Nasonia*, females produce large numbers of virtually identical offspring in a single clutch, making them ideal for a study of reaction norms. *Nasonia vitripennis, N. giraulti* and *N. longicornis* are parasitic wasps that parasitize various species of blow flies. I tested two strains of each of the three species for differences in emergence time, proportion of males and tendency to diapause due to different temperature environments. I also tested for genetic differences among the strains and any G by E interactions that might occur. I found that emergence time was the same for all species and all strains tested; as temperature increased the emergence time decreased. The proportion of males per clutch tended to have less of a plastic response, but there is evidence of a genetic response, with strains of the same species behaving

differently. Diapause showed more genetic than temperature variation. In

general, the G by E responses were modest.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

Temperature has been demonstrated to induce phenotypic plasticity in numerous insects for numerous traits (Gupta and Lewontin, 1982; Antolin, 1992; Windig, 1994; Delpuech et al, 1995; Brakefield et al, 1998; Van't Land et al 1999; Lysyk, 2001). A few examples of morphological traits exist. Ventral eyespots on the wings of the African butterfly *Bicyclus anynana* have shown to develop differentially due to temperature, large eyespots developing at cooler temperatures (Windig, 1994; Brakefield et al, 1998). Bristle number in *Drosophila pseudoobscura* has been shown to vary with temperature, the highest number being produced at an intermediate temperature (Gupta and Lewontin, 1982). Antolin (1992) found that increasing temperature and day length led to a more female biased sex ratio in the parasitic wasp *Muscidifurax raptor*.

In most cases life-history traits have been the ones examined in relation to temperature. In *Drosophila*, sex ratio (Van't Land et al 1999), developmental time (Gupta and Lewontin, 1982; Van't Land et al 1999) and ovary size (Delpuech et al, 1995) have all been studied for temperature effects. While temperature was not found to have a significant effect on sex ratio in *Drosophila* (Van't Land et al 1999), both developmental time and ovary size show a plastic response but no genotype-by-environment (G by E) interaction. In the vast majority of cases a decrease in temperature causes a slower developmental

1

time for ectotherms (Berrigan and Charnov, 1994). These life-history responses to temperature can be examined with analyses that test for G by E interactions and display norms of reaction; thus telling us if different genotypes behave differently in different temperature environments.

An organism of a specific genotype is said to exhibit phenotypic plasticity if it shows variation in a trait or traits that is dependant on changes in the external environment (Pollard et al, 2001). A reaction norm is the function that relates the environments to which a particular genotype is exposed and the phenotypes that can be produced by that genotype (Stearns and Koella 1986; Berrigan and Charnov 1994; Pigliucci, 2001). It is useful to plot reaction norms because they can show whether an organism is plastic and if it shows a genotype-by-environment interaction. G by E interactions measure how genotypes vary in their reaction norms (Antolin, 1992; Guntrip and Sibly, 1998); a strong G by E interaction would indicate that genotypes do not have parallel phenotypic responses across different environments.

Only a few animal species are suitable for studies of reaction norms. This is because the same genotype needs to be exposed to several environments (Gupta and Lewontin, 1982). This is easily accomplished in plants that can be cloned readily (Gupta and Lewontin, 1982), but is difficult in most sexually reproducing animals. One way to achieve the necessary replication is to use inbred strains in which genotypes can be easily replicated and distinguished. Another method is to use full-sib and half-sib groups that are split across environments, then take a mean value to evaluate plasticity across

environments (Scheiner, 1993). These methods will work for animals with short generation times and clutches that are large enough to provide the number of replicates needed to run the tests.

In the genus *Nasonia,* females produce large numbers of offspring in a single clutch, making them ideal for a study of reaction norms. Because the wasps mate within the host or almost immediately after emergence (Drapeau and Werren, 1999), they mate either with a sibling or close relative. The use of isofemale lines increases the value of *Nasonia* for studies of reaction norms because such lines provide stocks that contain virtually genetically identical individuals.

Nasonia vitripennis and the more recently discovered species, *N. giraulti* and *N. longicornis,* are small wasps that are parasitoids of cyclorrhaphous flies (flies that eclose from the puparium by a circular opening) (Darling and Werren, 1990). These three are the only species in the genus, with *N. giraulti* and *N. longicornis* apparently both being derived from *N. vitripennis* (Campbell et al, 1993). *Nasonia vitripennis* is a host generalist, using a wide range of flies in a variety of locations from birds' nests to animal carcasses (Whiting, 1967), while *N. giraulti* and *N. longicornis* specialize on the pupae of blowflies found commonly in birds' nests (Darling and Werren, 1990). *Nasonia giraulti* and *N. longicornis* are allopatric with each other. They are both found in sympatry with the cosmopolitan *N. vitripennis* in the northeastern and western United States respectively. Often, *N. vitripennis* has been found together with one of the other species in an individual bird's nest (Darling and Werren, 1990). The three

3

species can be distinguished by morphological and behavioral criteria. Morphologically they are most readily distinguishable by the wing length of the male (Darling and Werren, 1990): *Nasonia vitripennis* males have wings that are brachypterous and non-functional whereas *N. giraulti* males have longer broader wings, similar to those of the female, and *N. longicornis* males have intermediate wing sizes (van den Assem and Werren 1994). Females are a rich dark green color, have dark antennae and tend to be larger than males. Males on the other hand are a bright metallic green with light antennae and legs and tend to be smaller (Whiting, 1967). Males of *N. vitripennis* have vestigal wings and therefore cannot disperse by flying, however there is some evidence of dispersal in *N. giraulti* and *N. longicornis* (Drapeau and Werren, 1999). The three species show differences in their courtship behavior (van den Assem and Werren, 1994).

Under standard laboratory conditions of 24° C, 16h light : 8h dark cycle the wasps have a 16 – 18 day developmental time. There are four larval instars with facultative diapause occurring after the fourth instar (Saunders, 1965; Whiting, 1967). The adults eclose from their own puparia and remain in the host puparium anywhere from several hours to a day before emerging (Whiting, 1967).

The temperature in which *N. vitripennis* larvae are reared has been reported to affect the time that it takes them to develop (Whiting, 1967). As the temperature is increased, emergence time decreases as is expected in a poikilotherm. The probability of diapause, a state of suspended development, is

4

sensitive to the temperature of maternal development in *N. vitripennis* (Schneiderman and Horwitz, 1958; Saunders 1965; Whiting, 1967). Females reared in lower temperatures have an increased likelihood of producing diapausing larvae (Saunders 1965). When the maternal temperature was held constant and their larvae were reared at different temperatures, the incidence of diapause was not affected by changes in temperature (Schneiderman and Horwitz, 1958; Saunders 1965).

Like all wasps, *Nasonia* species have a haplo-diploid sex determination, and the sex of the progeny is under the behavioral control of the mother (Drapeau and Werren, 1999). The proportion of males per clutch has been shown to increase with superparisitism (more than one female parasitizing a host) (Werren, 1987). I did not have an apriori reason to expect that the proportion of males would be sensitive to temperature differences in *Nasonia*, but as previously mentioned, Antolin (1992) found an increase in sex ratio with increased temperatures in the parasitic wasp *M. raptor*. This result led me to predict that I would see a change in the proportion of males with temperature in *Nasonia*.

I hypothesized that the traits of emergence time, the proportion of males per clutch and the tendency to diapause would show plasticity in response to temperature. I based this hypothesis on the previous studies done on *N. vitripennis* and the plasticity that had been demonstrated. I predicted that I would see differences between the three species in their response to the different temperature environments; thus demonstrating a G by E interaction. I

5

also predicted that within each species, the strains that I used would have similar responses to the temperature environments, thus giving no G by E response. My reasoning behind this was that the different species, being more genetically different, would be more likely to have different responses to temperature than two strains of the same species which are more closely related.

Because of the known maternal influence on diapause, I was particularly interested to see if either of the other two traits that I tested could be altered due to the maternal temperature, independent of the larval temperature. I predicted a maternal influence on diapause and the proportion of males, but not for emergence time. I expected that emergence time would not be altered by maternal temperature. In previous studies of ectotherms it has been the larval temperature that affects the developmental time (Schneiderman and Horwitz, 1958; Saunders, 1965; Whiting, 1967; Gupta and Lewontin, 1982;).

Chapter 2

Methods

Standard Rearing

The six strains of *Nasonia* (two strains of each species) had been
collected in different parts of the United States and were provided by Dr. J.
Werren at the University of Rochester. The *N. vitripennis* stock strains were
collected in Northern Ohio in 1989 (NVOH 204; or NV1) and Nevada in 1991
(NVXNVB 401AF; or NV2). The *N. giraulti* stocks were from Pennsylvania in
1989 (NGPA 233F2; or NG1) and New York in 1987 (RV2; or NG2). Lastly, the
N. Longicomis strains were from northern Utah in 1989 (NLUT 218; or NL2) and
northern California in 1990 (NLCA 003270A; or NL1). All stocks had been
maintained in diapause before being shipped to us (Werren, personal
communication).

These stocks were maintained in our laboratory as active, non
diapausing stocks. In maintaining the stocks, I placed 5-7 mated females in a
vial with five hosts (*Sarcophaga* pupae) and allowed the females to lay eggs.
Five vials of each strain per generation were reared to insure that there would
be enough progeny for the strains' persistence. The vials were kept in an
incubator with a temperature of 24° C with a 16h:8h light:dark light cycle.
Relative humidity was kept between 50% and 60%.

Traits Scored

To score emergence time I checked the vials daily at the same time of day and recorded which vials (if any) had wasps that were starting to emerge from the host puparium. The wasps eclose, that is come out of their own pupa, about a day before emergence from the host. The delay is apparently due to the time needed to chew their way out of the host (Whiting, 1967). Therefore, in *Nasnoia*, developmental time can be inferred from emergence time.

Once all of the wasps had ample time to emerge (at least a week after the first wasp emerged), I dissected each host under a dissecting microscope to find remaining wasps and larvae. I counted the males, females, and diapausing larvae. Males and females were separated based on the physical differences between the sexes mentioned earlier. Diapausing larvae were classified by the semi-transparency of their fat body and the possession of a sticky waxy coating (Whiting, 1967). I never found any larvae still in the host that were not in diapause.

I calculated the total clutch size, the percent of diapausing larvae and the proportion of males in each clutch. A clutch was defined as the total number of progeny from one host. The tendency to diapause was defined as the percentage of the entire clutch that was in diapause. The proportion of males was calculated from adults only because the sex of the larvae cannot be determined by visual inspection. Not all hosts provided usable data in all categories, because in some cases the entire clutch was in diapause or the host had not been parasitized.

8

Overview of Experiments

My preliminary experiment helped me to determine the methods for my main study of temperature effects. The data from preliminary experiment were useful in interpreting the data from the main experiment but will not be given detailed treatment on their own. The main temperature effect experiment addresses the rearing temperature of the larvae only; the mothers and larvae had the same environment. In the second, maternal influence experiment, the temperature of the larvae and the mothers were different, so that I could test for maternal influences on the traits in question. I did not change the temperature of an incubator, so there is a possibility that results are due to the idiosyncrasies of the chambers. This appears to be unlikely.

Preliminary Experiment

In this study the same methods were employed as in the temperature effect study described below, except that one female per host was used. This study had been used to help me design the main study, but later I found the results to be useful in deciding whether certain effects were due to temperature or superparasitism (see Discussion).

Experiment 1: Temperature Effect

In this experiment , mothers and larvae were raised in the temperature in which their progeny would be developing to control for any possible maternal influences. For each strain, I reared waspss in three incubators at three

different temperatures: 21°C, 24°C, 28°C, with the light cycle and relative humidity set as above. These temperatures were chosen to bracket the standard rearing temperature of 24°C. I placed two females in a vial with a host (*Sarcophaga* pupa) for 24 hours at the specified temperature. I then removed the host and put it in a separate labeled vial at the specified temperature and provided another host to the females, again for 24 hours. This procedure gave me two clutches from the same females. Ten replicates (A-J) were established at each temperature, however, if a female died I omitted the entire replicate. The total sample was 360 (10 pairs of females x 2 clutches per female pair x 3 species x 2 strains per species x 3 temperature).

Superparasitism usually increases the proportion of males per clutch (Werren, 1987; King and Skinner, 1991; Antolin 1992), but I thought it best to put two females on each host for a greater chance of getting usable data. My idea for this method came from a previous study of a wasp confamilial to *Nasonia*, *Spalangia cameroni*; in this study paired females were found to produce a significantly greater proportion of sons than lone *S. cameroni* females (King, 1996) These wasps were taking cues from other females being present and altering their behavior. In early pilot tests I found one strain (NL2) was producing almost 100% diapausing larvae. Due to the fact that this same strain was producing non-diapausing larvae regularly when maintained with multiple females in the breeding vial, I thought that like *S. cameroni*, ovipositing NL2 females might be taking cues from other wasps nearby. Putting two

10

females on a host did help to produce useful data; I used this method for all strains.

Experiment 2: Maternal Influence

In this study I wanted to learn if the temperature in which the mother was reared influenced the three traits, emergence time, the proportion of males per clutch and the tendency to diapause. The same stocks were used and the same variables were tested as above. In this procedure, three females that were raised at 21° C were placed on three hosts in a single tube for 24 hours in the 21° C incubator. After 24 hours the hosts were replaced with three new hosts to obtain another clutch. When the hosts were removed they were separated and placed in the three different incubators set at the same temperatures used previously, 21°, 24° and 28° C. They were labeled as coming from a female that was raised in the 21° C incubator. The procedure was repeated for all strains at all temperatures (Figure 1 – All figures and tables are in the Appendix).

The same methods were employed to check for emergence time, the proportion of males, and the tendency to diapause. The total sample was 2160 (20 female groups x 2 clutches per female group x 3 species x 2 strains per species x 3 maternal temperature x 3 larval temperature).

Analysis

In order to best view the data, the means of each genotype were plotted across the three temperature environments. This allowed me to look for plasticity and G by E effects. Using a univariate ANOVA I tested for significant effects of temperature, species, and strain within species (hereafter strain) as well as for G by E interactions between the temperature environments and genetic factors due to species and strain. Because I measured multiple traits on the same animals I did a sequential Bonferroni correction on the data.

Rather than simply basing my conclusions on what the statistical tests told me, I have chosen to look at the data for biological significance. Probabilities are not always the best way to interpret data as they are a function of the sample size, they can get smaller with a larger sample (Pigliucci, 2002). It is important to look at the effect size (the value of the means square) of a factor. First, it does not change with the sample size, and second, it tells how strong the influence of a certain factor is on the dependant variable (Pigliucci, 2002). This number can be more useful than a probability when interpreting biological data.

Chapter 3

Results

Sample Sizes

Sample sizes were measured by clutch in these studies, one unit was one clutch. The maximum sample size was 40. Sample sizes are variable throughout the studies (Tables 1, 2, and 3) due to females not laying eggs or complete diapause of the clutch. However, there are only 7 cases total with fewer than five data points

Preliminary Experiment

The results of the preliminary experiment are shown in Figure 2. These results were useful in interpreting the results from the main experiments and will be considered in the Discussion.

Temperature Effect

Emergence time was highly plastic with regard to temperature, but not highly variable between strains or species (Figure 3). As the temperature increased the emergence time for all strains decreased significantly (Figure 3). The effect of temperature was nearly 30 times that of next closest factor (Table 4a). Although there are significant differences for strain and species, they do not have as strong an effect on developmental time as temperature does. The interactions between temperature and species and between temperature and strain test for a G by E effect, they are significant but the effect sizes are very

13

small and may not be biologically significant. *Nasonia longicornis* and *N. giraulti* showed no variation in their reaction norms for developmental time. Thus the interaction effects for emergence time must be due to the *N. vitripennis* strains (Figure 3b). However, because the difference in developmental time was less than a day, with the error it is most likely not an important G by E interaction.

The proportion of males per clutch gave somewhat different results (Figure 4). The overall temperature effect was very weak. The strongest effect came from strain (Table 4b). The plot of reaction norms (Figure 4), shows that this effect is largely due to *N. vitripennis*. On inspection it can be seen that only one strain (NV1) is likely to contribute to the significance of the result (Figure 4). There was no interaction effect between temperature and species (Table 4b). Because of the unique response of NV1, I asked whether any replicates had an all male clutch (indicating that an unmated female had been used) that might be skewing the data. I found only one case and after I eliminated this data point I found no difference in the shape of the plot or in the significance of the tests. Thus, this trait is plastic in one or two strains and the data suggest that *N. vitripennis* shows G by E for it.

The results for diapause were the most surprising. At least one strain from each species showed the lowest level of diapause at lower temperatures (Figure 5). Again all factors have significant effects (Table 4c), with the strongest effect being due to the differences between strains. The strain effect is attributable to the large difference between the *N. longicornis* strains (Figure

14

5c). The effect of species is stronger than the effect of temperature or either interaction. The plot of reaction norms for this character across species (Figure 6), shows that the species behaved differently across temperatures: *N. vitripennis* showed consistently low diapause, *N. giraulti* had higher diapause at higher temperatures, and *N. longicornis* had lower diapause at higher temperatures. Only *N. longicornis* had a greater probability of diapause at the low temperature (Figure 6c); this difference from the other species could have caused the significant G by E interactions at the species level.

Maternal Influence

In this study I wanted to learn if the temperature in which the mother was reared influenced the three traits, emergence time, the proportion of males per clutch and the tendency to diapause.

Emergence time showed the same trend of decreasing with increasing temperatures (Figure 7) as it did in the previous study. All of the factors were significant, but the temperature in which the larvae were raised showed the largest effect size by far (Table 5a), nearly 55 times that of the next largest effect. At the three larval temperatures, all strains appeared to behave the same regardless of the maternal temperature (Figure 8), however the effects of strain and species were the second strongest effects (Table 5a) indicating that the two different strains within each species used in this study behaved differently with regards to emergence time. The plots (Figure 7) show that this is in fact the case. The clearest example here is in Figure 7c with the

15

differences of the plots between the two *N. giraulti* species, but even this difference is modest. The effects of the interactions here are so miniscule (Table 5a) that the statistical significance shown here may not indicate an important G by E interaction.

The proportion of males was only slightly affected by maternal temperature, alone and in interactions (Table 5b). Although there are no large statistical effects, the largest effect for the proportion of males is that of strain (Table 5b). In this case the effect was almost three times as large as the next closest one (Table 5b). Strain differences are very apparent here, particularly for *N. vitripennis* (Figure 9). The plots (Figure 9) show that all of the lines, with the exception of two of the *N. giraulti* plots, seem to follow the same general pattern of little change across the temperature environments. This means that while maternal temperature did have a significant effect (Table 5b), it was not very strong. The interactions of maternal temperature with species and with strain have a very small effect size (Table 5b).

Once again, the tendency to enter diapause was the most interesting trait (Table 5c, Figure 10). All main effects were significant; the strongest effect came from strain, which was nearly 5 times that of the next closest factor. The plots (Figure 10) illustrate the strain effects. Maternal temperature had a significant effect on the tendency to enter diapause (Table 5c), but it is clearly not the strongest or the most significant effect: the effect sizes of the larval temperature, species and strain are far larger. This can also be seen in the plots because if maternal temperature had a stronger effect, the majority of the

16

reaction norms would have less variation across the larval temperature

environments (Figure 10). Of the significant interactions, the ones involving

larval temperature have a very small effect size; interactions involving maternal

temperature were stronger despite maternal temperature's small role as a main

effect.

Chapter 4

Discussion

Emergence time, the proportion of males, and the tendency to diapause have all been demonstrated to show plasticity due to environment stimuli (Saunders, 1965; Whiting, 1967; Werren,1987; Antolin, 1992). Temperature has a profound influence on various insect life history processes such as development and reproduction: as temperature increases, developmental time and reproduction age decrease (King and Skinner, 1991; Antolin, 1992; Van't Land et al., 1999; Lysyk, 2001). In my study temperature was used as the environmental stimulus to test for plasticity in these three traits. I hypothesized that all traits would show plasticity in response to temperature and that strains within species would show the same pattern of plastiscity; thus no G by E interactions would be evident within species. However, I predicted that G by E interactions may be evident between species mostly due to the fact that two of the species are allopatric (*N. longicornis* and *N. giraulti*) but also because the three species should be more genetically diverse than the strains within a species. Below I discuss each trait in turn.

Emergence Time

Natural populations of *Drosophila melanogaster* show a latitudinal cline for developmental time (Van't Land et al., 1999). Populations collected closer to the equator (warmer environments) develop more slowly. However, in

18

laboratory stocks the opposite was shown in that development was slower in cooler temperatures (Van't Land et al., 1999). In *Muscidifurax raptor*, a wasp that is confamilial with *Nasonia*, developmental time was shown to decrease as temperature increased (Lysyk, 2001). For *Nasonia vitripennis*, the time spent in development has been reported to vary inversely with the temperature of the environment (Whiting, 1967; Darling and Werren, 1990). This is not surprising because metabolic rates generally increase with increased temperature in insects, within normal temperature ranges.

My results are consistent with these earlier results (Table 4a, Figure 3). All three species of *Nasonia* showed significant plasticity of emergence time in response to temperature. The species and strains differed significantly for this trait, but the effect sizes were miniscule in comparison to that of the temperature effect (Table 4). The plots do not indicate a genetically-based difference in emergence time between species or strains in *Nasonia's* response to temperature (Figure 3). Plots of reaction norms for this trait among species (Figure 6), show that *N. giraulti* and *N. longicornis* follow the same general trend across temperatures and that the significant difference between species is due to *N. vitripennis*; when one accounts for the small effect size, the difference might be biologically meaningless.

When I tested for a maternal influence on emergence time, I found strong support for the effects of larval temperature and little support for effects of maternal temperature and interactions (Table 5a). I believe that the support for maternal influence and G by E interactions could be determined with more

19

confidence were this study done again with slightly different methods. The studies reported here are based on data that were collected every 24 hours; in future studies, it might be beneficial to check for emergence every 8 or 12 hours. This could provide greater resolution and test more clearly for a G by E interaction for emergence time.

Proportion of Males

Local mate competition (LMC) is the most commonly cited mechanism for a female biased sex ratio (Antolin, 1992). LMC is possible when related individuals develop in close proximity, such as a group of sibling parasitoids within a host. Because these local groups of individuals are isolated, they have a high probability of mating with their siblings, creating no competition to transmit their mother's genes. Only a few males are required to successfully inseminate all of their sisters. Therefore, under LMC conditions a female biased sex ratio is favored because increasing the number of sons would not greatly increase the number of grandchildren (Hamilton, 1967; Werren, 1987). The proportion of sons is predicted to increase with the number of foundresses (King and Skinner, 1991), as well as with female abilities to detect previous parasitism, superparasitize, and alter sex allocation during oviposition (Antolin, 1992). Much attention in sex ratio research has focused on parasitic wasps because of their haplo-diploid sex determination (Werren, 1987; Antolin, 1992). This system allows for precise female control of the sex ratio because the

decision to fertilize an egg is under control of the ovipositing female (Werren, 1987; Antolin, 1992).

Temperature could affect sex ratio (proportion of males in the clutch) in several ways. First, a female could respond to temperature by altering the sex ratio of her offspring. Second, the rate of superparasitism could change with temperature, if females are more likely to lay eggs at some temperatures. Third, differential temperature-dependant mortality of male and female larvae could cause a shift towards or more or less female biased sex ratio. Below I address these possibilities.

In this study, the proportion of males did not change in response to temperature in any strain (Figure 4). With the exception of NV1, all plots appear to be flat. The significance of the largest effect, strain, appears to be due to *N. vitripennis* (Figure 4). To test the possible role of strain, I examined my preliminary data that came from tests in which I used one female per host. The experiments that used one female yielded the same results, with strain showing the strongest effect ($F_{3, 208}$ = 44.980, P<.001). Regardless of the number of mothers, NV1 had a high proportion of males (Figures 3, 10). This shows that the differences between the strains of *N. vitripennis* were not sensitive to superparasitism. Thus it appears that *N. vitripennis* harbors genetic variation for a difference in sex ratio.

Because the sex of the offspring is controlled by the ovipositing female (Whiting, 1967; Werren, 1980) the environment of the larva could affect proportion of males if one sex of the larvae was tolerant of certain

21

temperatures. The lack of an effect of the larval temperature suggests that there is no differential mortality among the larvae.

Diapause

Both in nature and in the laboratory a persistent, irregular proportion of *N. vitripennis* larvae enter diapause (Schneiderman and Horwitz, 1958). In most species in which diapause occurs, it can be considered an adaptation to synchronize the life cycle of the animal with the environment so that active stages will occur during the most favorable season (Whiting, 1967). Food, photoperiod, temperature and maternal influences have all been demonstrated to stimulate diapause (Lees, 1955). As a rule, high temperatures tend to avert diapause while low temperatures favor its initiation (Mousseau and Dingle, 1991; Lees, 1955). This has been reported in *N. vitripennis* as well (Schneiderman and Horwitz, 1958; Whiting, 1967).

Diapause might be induced by the mother as a result of the environment that she experiences, as has been shown in the lepidoptera *Bombyx mori* and *Orgyia thyellina* (Mousseau and Dingle, 1991; Kimura and Masaki, 1977), and the parasitoid wasp *Trichogramma evanescens,* and *N. vitripennis* (Schneiderman and Horwitz, 1958; Zaslavsky, 1982). It might also be induced directly in the larva by the condition that it experiences. In the published cases involving *N. vitripennis,* temperature was found to influence the maternal generation and not the offspring (Schneiderman and Horwitz, 1958; Whiting 1967). Offspring of females raised in higher temperatures had a lesser

22

tendency to diapause than those of females raised in lower temperatures (Schneiderman and Horwitz, 1958; Saunders, 1965; Whiting, 1967). When larvae were tested independent of their mother's rearing temperature there were no factors that directly induced diapause (Schneiderman and Horwitz, 1958; Saunders, 1965). Chilling the female while her eggs were developing increased the likelihood that she would produce larvae that enter diapause (Schneiderman and Horwitz, 1958; Saunders, 1965). Although the temperature history of the ovipositing female was found to be the most critical factor in inducing diapause, it may not be the only factor because diapause is found across all temperatures (Schneiderman and Horwitz, 1958). One demonstrated factor is day length (Saunders, 1965). In contrast to the proportion of males, diapause has been shown to be unaffected by the number of females that parasitize a host (King and Skinner, 1991).

The effects of temperature in my study are inconsistent with the results reviewed above (Lees, 1955; Schneiderman and Horwitz, 1958; Saunders, 1965). The temperatures that I used (21° C, 24° C and 28° C) were different from those used in the previous studies (10° C, 15° C and 25° C). *Nasonia* parasitize hosts in bird's nests in mid to late spring, shortly after the young have fledged. So, instead of cooler spring temperatures, hot summer temperatures may indicate an unfavorable environment for *Nasonia*, because hosts would be unavailable. More extreme cool temperatures, such as the 10° C used by Schneiderman and Horwitz (1958) could also serve to induce diapause for clutches of individuals that did not diapause over the hot temperatures.

23

The species and strains of *Nasonia* that I tested showed different tendencies to diapause across temperatures: as temperature increased, the tendency to diapause was constant or increased. With the exception of one *N. longicornis* strain (NL2) I found *Nasonia* to be less likely to diapause at the lower temperature (Figure 5, Table 4c). The most dramatic of these results is in *N. giraulti*. Even though temperature influenced diapause, it was not the strongest factor: strain had more than three times the effect of temperature (Table 4c). The effect of strain is largely due to the very different reaction norms of the two *N. longicornis* strains (Figure 5).

The test for a maternal influence on diapause provided additional insights (Table 5c, Figure 10). While NV1 followed the published trend in *N. vitripennis*, less diapause at higher maternal temperatures (Schneiderman and Horwitz, 1958; Saunders, 1965), larval temperature showed a slightly stronger effect on diapause than maternal temperature did (Table 5c). As with the results from the proportion of males, strain had the greatest effect on diapause: regardless of temperature the strains behaved differently from each other. NL2 appeared to have a very high tendency to diapause while NL1 showed an opposite trend of little diapause (Figures 9 and 11). NV1 repeatedly showed less diapause than NV2 (Figures 9 and 11). The *N. giraulti* strains showed no trends of consistent high or low diapause at all (Figures 9 and 11). Because the strains show such large differences even within species, any differences between species appear to be attributable to the strain differences. *N. giraulti* may show G by E interactions because the strains have very different reaction

24

norms in response to maternal temperatures as well as at different larval temperatures. A surprising effect is the three way interaction of maternal temperature, larvae temperature and strain (Table 5c). Again, this appears to be because of *N. giraulti* (Figure 10).

In *N. vitripennis,* Schneiderman and Horwitz (1958) showed that chilled females at 10° C produced diapausing larvae, but Moursi, using females chilled at 11° C (1946b) did not. The different results might be because the two experiments were conducted on different strains (Schneiderman and Horwitz, 1958). My results also indicate the importance of genetically based differences in the tendency to diapause although the largest difference between strains that I detected for diapause was within *N. longicornis.*

Comparing Results Across Studies

It is interesting to compare the plots for the three studies that I conducted; the main analysis of temperature effects, the preliminary study (Figure 2) and the analysis of maternal influence (Figure 12). I would expect these plots to be similar.

The plots for emergence time were very similar; there were slight differences in the position of the strains, but I believe that this can be explained by the method of sampling that was employed in this study. That is, if emergence was measured over shorter periods of time a more accurate measure of emergence would be taken and the differences would most likely be stronger if temperature has a strong influence.

For the proportion of males, NV1 was high in all of the studies. Th strains show little sensitivity to temperature for this trait except for NL2 in the preliminary study. However, when I looked at the sample size for this odd data point (Figure 2 NL2 at 21° C) I found that it was a sample of one (Table 2). The reason for this small sample size was the high tendency of NL2 larvae to go into diapause when only one female was placed on a host. When clutches yielded no adults it was impossible to score the proportion of males. Thus the NL2 data may not be inconsistent across experiments.

The tendency to diapause shows the most variation between experiments. *N. giraulti* showed almost the same reaction norms for the preliminary study as it did for the study of temperature effects. When comparing those results to those of the maternal influence study they were similar, both strains had low diapause at the low temperature with significantly more diapause at the higher temperature. The largest discrepancy here was NL2: in both the temperature effect and preliminary studies it showed a high incidence of diapause that decreased as the temperature increased. In the maternal influence study its reaction norm plot (Figure 12) gave more of a 'V' shape with lower diapause at 24 C. In these cases I found the sample sizes to be quite adequate, at least 19 (Table 3). This suggests that some other, unmeasured factor affects the tendency to diapause in the different species of *Nasonia*.

Future Directions

In this discussion I have repeatedly shown that a high statistical significance for an effect can be accompanied by a small effect size making the true biological significance questionable (Pigliucci, 2002). The uncontrollable factors of maternal egg-laying and the tendency to diapause resulted in different sample sizes with almost every factor of every trait that I examined. Because the effect sizes allowed me to identify the strongest and weakest influences on the traits that I studied, in future studies the traits that are most important biologically can be examined further.

In future studies it would be beneficial to collect data at more extreme temperatures. In these studies I used temperatures with a range of 7°C. Using more extreme temperatures may show stronger temperature effects. Expanding the study to include four or five strains per species would make it easier to detect genetic differences between species. In this study I was hesitant to draw any conclusions about the genetic differences of species because in most cases the two strains of each species exhibited different reaction norms across the three temperatures.

It would also be valuable to collect field data on temperature effects. Tests done in the laboratory on inbred lines may not always hold true in the field. However, collecting field data for *Nasonia* would not be easy in a study such as this. The wasps are very small (1-3mm) and not easy to find or to identify without the use of a microscope. If a bird's nest containing host pupae is found, it could be up to two weeks before learning if there are wasps in the

pupae. Labeling, tracking and observing the wasps is not possible because of their size.

Some data could be collected in the field. First, the temperatures and relative humidities in bird's nests where *Nasonia* are found can be recorded. This information would tell us the preferred range of temperatures in which *Nasonia* parasitizes flies and suggest a range of temperatures to use in future studies. Second, after emergence, the parasitized hosts can be collected and brought to the lab to see if they contain any diapausing larvae. This method would not allow for calculation of percentage of diapause, but would indicate whether diapause occurs in that environment. Finally, if a nest was found with emerging wasps, a sample could be taken to test for relatedness within a nest. This information would give a better idea on the degree of relatedness of wasps in the same host and what this means for LMC and temperature-dependent sex ratios. The traits measured in my studies would be impossible to study naturally in the field. However, if a device were built that would contain the wasps upon emergence that imitated the conditions in a bird's nest and placed in an area where wasps had been collected; it might be possible to collect the data that would be needed. The stocks used in such a study would need to be wild caught or as close to this as possible. With careful field collection and a sound experimental design, field data could significantly add to the information that we have on *Nasonia*.

Plasticity

The genus *Nasonia* shows plasticity in response to the larval temperature. The response of Emergence time to temperature was the same for all species and all strains tested: as temperature increased the emergence time decreased. Of the factors that I tested, temperature appears to be the main one influencing this response, because it not only shows strong significance but also a large effect size. The proportion of males per clutch tended to have less of a plastic response, but there is evidence of a genetic response, with strains of the same species behaving differently. A reliable test of differences between species would require the analysis of more strains, because the two strains behaved so differently. Diapause, like the proportion of males per clutch, showed more genetic than temperature variation. In general, the G by E responses were modest.

29

BIBLIOGRAPHY

Antolin, M. (1992). Sex ratio variation in a parasitic wasp I. Reaction norms. Evolution **46**: 1496-510.

Berrigan, D and E. Charnov . (1994) Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. Oikos **70**: 474–478.

Brakefield, P.M., F. Kesbeke and P.B. Kock. (1998). The regulation phenotypic plasticity of eyespots in the butterfly *Bicyclus anynana.* American Naturalist **156**: 853-860.

Campbell, B.C., J.D. Steffen-Campbell, and J.H. Werren. (1993). Phylogeny of the *Nasonia* species complex (Hympenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 288 rDNA sequences. Insect Molecular Biology **2**: 225-237.

Darling, D.C. and J. Werren (1990). Biosystematics of *Nasonia* (Hymenoptera: Pteromalidae): Two new species reared from birds nests in North America. Annals of the Entomological Society of America **83**: 352-370.

Delpuech, J., B. Moreteau, J. Chiche, E. Pla, J. Vouidibio, and J. R. David. (1995). Phenotypic plasticity and reaction norms in temperate and tropical populations of *Drosophila melanogaster.*Ovarian size and developmental temperature. Evolution **49**: 670-675.

Drapeau, M., and J. Werren. (1999). Differences in mating behavior and sex ratio between three sibling species of *Nasonia.* Evolutionary Ecology Research **1**: 223-234.

Guntrip, J., and R. M. Sibly (1998). Phenotypic plasticity, genotype-by-environment interaction and analysis of generalism and specialization in *Collosobruchus maculatus.* Heredity **81**: 198-204.

Gupta, A.,and R. Lewontin (1982). A study of reaction norms in natural populations of *Drosophila pseudoobscura.* Evolution **36**: 934-948.

Hamilton, W.D. (1967). Extraordinary sex ratios. Science **156**: 477-488.

Kimura, M. T., and S. Masaki. (1977). Brachypterism and seasonal adaptation in *Orgyia thyellina* Butler (Lepidoptera, Lymantriidae). Kontyu **45**: 97-106

King, B. H. (1996). Sex ratio responses to other parasitoid wasps: multiple adaptive explanations. Behavioral Ecological Sociobiology **39**: 367-374.

King, B.H., and S. Skinner. (1991). Sex ratio in a new species of *Nasonia* with fully winged males. Evolution **45**: 225-228.

Lees, A.D. (1955). The physiology of diapause in arthropods. Cambridge University Press.

Lysyk, T. (2001). Relationships between temperature and life history parameters of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). Environmental_Entomology **30**: 147-156.

Pigliucci, M. In press. Studying the plasticity of phenotypic integration in a model organism in M. Pigliucci, ed. The Evolutionary Biology of Complex Phenotypes. Oxford University Press, Oxford.

Pigliucci, M. (2001) Phenotypic plasticity: Beyond nature and nurture. Johns Hopkins University Press.

Pollard, H., M. Cruzan, and M. Pigliucci. (2001). Comparative studies of reaction norms in *Arabidopsis* I. Evolution of response to day length. Evolutionary Ecology Research. **3**: 129-155.

Moursi, A.A. (1946). The effect of temperature on development and reproduction of *Mormoniella vitripennis* (Walker). Bulletin of the Society of Fouad Entomology. **15**: 39-61

Mousseau, T., and H. Dingle. (1991). Maternal effects in insect life histories. Annual Review of Entomology. **36**: 511-534.

Saunders, D.S. (1965). Larval diapause of maternal origin: induction of diapause in *Nasonia vitripennis* (Walk) (Hymenoptera: Pteromalidae). Journal of Experimental Biology. **42**: 495-508.

Schneiderman, H.A., and J. Horwitz. (1958). The induction and termination of falcultative diapause in the chalcid wasps *Mormoniella vitripennis* (Walker) and *Tritneptis klugii* (Ratzeburg). Journal of Experimental Biology. **35**: 520-551.

Scheiner, S. (1993). Genetics and evolution of phenotypic plasticity. Annual Review of Ecology and Systematics. **24**: 35-68.

Stearns, S.C., and J.C. Koella. (1986). The evolution of phenotypic plasticity in life history traits: predictions os reaction norms for age and size at maturity. Evolution **40**: 893-913.

van dem Assem, J., and J. Werren (1994). A comparison of the courtship and mating behavior of three species of *Nasonia* (Hymenoptera: Pteromalidae). Journal of Insect Behavior **7**: 53-66.

Van't Land, J., P. Van Putten, B. Zwaan, A. Kamping, and W. Van Delden (1999). Latitudinal variation in wild populations of *Drosophila melanogaster*. Heritabilities and reaction norms. Journal of Evolutionary Biology **12**: 222-232.

Weis, A.E. and Gorman, W.L. (1990). Measuring selection on reaction norms: An exploration of the *Eurosta – Solidago* system. Evolution **44**: 820-831.

Werren, J. (1980). Sex ratio adaptations to local mate competition in a parasitic wasp. Science **208**: 1157-1159.

Werren, J. (1983). Sex ratio evolution under local mate competition in a parasitic wasp. Evolution **37**: 116-124.

Werren, J. (1987). Labile sex ratios in wasps and bees. BioScience **37**(7): 498-506.

Whiting, A. R. (1967). The biology of the parasitic wasp *Mormoniella vitripennis* [=*Nasonia brevicornis*] (Walker). Quartely Review of Biology **42**: 333-406.

Windig, J. (1994). Genetic correlations and reaction norms in wing pattern of the tropical butterfly *Bicyclus anynana*. Heredity **73**: 459-470.

Zaslavsky, V. A., and T. Y. Umarova. (1982). Photoperiodic and temperature control of diapause in *Trichogramma evanescens* Westw. (Hymenoptera, Trichogrammatidae). Entomological Review. **60**:1-12.

APPENDIX

Table 1 Strain means for developmental time, proportion of males and % of clutch in diapause with two standard errors and sample sizes included. Results from the temperature effect study. See Figure 2 for legend definitions.

	Developmental Time				Proportion of males				Tendency to Diapause		
	21°C	24°C	28°C		21°C	24°C	28°C		21°C	24°C	28°C
N. vitripennis											
NV1	20.4+0.15 (17)*	16.2+0.37 (18)	12.8+0.64 (12)		0.21+0.04 (17)	0.32+0.70 (18)	0.39+0.14 (13)		0 (17)	0 (18)	5+3 (14)
NV2	22.4+0.26 (16)	17.9+0.13 (16)	12.5+0.31 (11)		0.11+0.02 (16)	0.15+0.02 (16)	0.14+0.02 (11)		16+10 (16)	10+07 (17)	28+12 (13)
N. giraulti											
NG1	24.4+0.26 (16)	18.0+0.00 (5)	14.2+0.33 (12)		0.13+0.05 (16)	0.15+0.04 (5)	0.12+0.04 (12)		3+4 (16)	65+12 (13)	37+9 (17)
NG2	22.4+.31 (16)	17.7+0.42 (6)	14.2+0.33 (6)		0.11+0.03 (16)	0.18+0.06 (6)	0.23+0.07 (6)		0.03+0.07 (16)	78+20 (13)	3+14 (17)
N. longicornis											
NL1	21.8+0.29 (16)	16.2+0.19 (19)	12.5+0.28 (14)		0.12+0.02 (16)	0.21+0.03 (19)	0.14+0.05 (14)		0 (16)	0 (19)	25+9 (17)
NL2	23.5+0.58 (4)	17.2+0.29 (9)	13.7+0.35 (13)		0.09+0.02 (4)	0.14+0.06 (9)	0.12+0.02 (13)		91+39 (16)	64+10 (18)	43+9 (13)

*numbers in parenthesis are sample sizes

Table 2 Strain means for developmental time, proportion of males and % of clutch in diapause with two standard errors and sample sizes included. Results from the preliminary study. See Figure 2 for legend definitions.

	Emergence Time				Proportion of males				Tendency to Diapause		
	21°C	24°C	28°C		21°C	24°C	28°C		21°C	24°C	28°C
N. vitripennis											
NV1	24.3+0.47 (9)*	16.2+0.52 (15)	12.0+0 (11)		0.27+0.05 (9)	0.29+0.06 (15)	0.27+0.08 (11)		47+18.2 (14)	22+6.4 (18)	1+1.2 (11)
NV2	22.4+0.24 (18)	17.9+0.15 (18)	12.4+0.24 (18)		0.10+0.01 (18)	0.13+0.02 (18)	0.10+0.01 (18)		8+8.0 (18)	4+5.4 (18)	9+8.2 (18)
N. giraulti											
NG1	24.6+0.24 (18)	17.6+0.37 (8)	14.3+0 (12)		0.10+0.01 (18)	0.14+0.02 (8)	0.11+0.02 (11)		4+4.6 (18)	54+11.6 (16)	59+4.0 (4)
NG2	24.9+.34 (16)	17.9+0.47 (10)	14.3+0.33 (8)		0.11+0.02 (16)	0.12+0.02 (10)	0.10+0.01 (8)		3.1+6.0 (17)	13+14.4 (18)	2.8+5.6 (14)
N. longicornis											
NL1	25.29+0.25 (14)	17.1+0.15 (18)	12.5+0.28 (14)		0.16+0.04 (14)	0.15+0.04 (18)	0.29+0.05 (14)		35+11.8 (19)	11+6.6 (19)	8+8.0 (14)
NL2	24+0 (1)	17.3+0.67 (3)	13.3+0.24 (15)		.29+0 (1)	0.10+0.10 (3)	0.14+0.03 (16)		99+0 (18)	88+2.0 (12)	27+6.8 (19)

* numbers in parenthesis are sample sizes

Table 3 Strain means for developmental time, proportion of males and % of clutch in diapause with two standard errors and sample sizes included for (A) maternal temperature of 21 C across the larval temperatures, (B) maternal temperature of 24 C across the larval temperatures and (C) maternal temperature of 28 C across the larval temperatures. Results from the maternal influence study. See Figure 2 for legend definitions.

A

		Developmental Time				Proportion of males				Tendency to Diapause		
		21°C	24°C	28°C		21°C	24°C	28°C		21°C	24°C	28°C
N. vitripennis												
	NV1	20.0+0.52 (25)*	16.4+0.18 (31)	13.3+0.20 (21)		0.19+0.07 (25)	0.25+0.06 (25)	0.23+0.06 (32)		20+3.2 (25)	1.0+2.0 (32)	21 (28)
	NV2	21.7+0.44 (22)	17.5+0.20 (31)	13.5+0.34 (25)		0.09+0.02 (22)	0.08+0.03 (31)	0.12+0.03 (25)		8+7.6 (23)	11+7.2 (32)	14+3.6 (28)
N. giraulti												
	NG1	24.4+0.32 (21)	17.2+0.32 (19)	14.9+0.38 (19)		0.13+0.03 (21)	0.12+0.04 (20)	0.13+0.02 (18)		1+1.0 (21)	0 (21)	0 (18)
	NG2	22.7+.19 (23)	17.9+0.28 (20)	14.5+0.24 (20)		0.13+0.04 (23)	0.17+0.04 (20)	0.13+0.04 (24)		19+1.8 (28)	6.0+8.4 (20)	17+6.0 (28)
N. longicornis												
	NL1	23.6+0.60 (30)	18.0+0.26 (31)	13.6+0.40 (32)		0.18+0.07 (30)	0.18+0.06 (31)	0.18+0.02 (33)		8+6.8 (31)	10.1+9.0 (20)	0 (33)
	NL2	24.5+0.30 (12)	16.8+0.38 (19)	14.0+0.40 (19)		0.01+0.01 (12)	0.05+0.02 (19)	0.08+0.02 (19)		73+10.8 (30)	70+13.2 (20)	69+10.8 (28)

*numbers in parenthesis are sample sizes

Table 3 Continued.

B

	Developmental Time			Proportion of males			Tendency to Diapause		
	21°C	24°C	28°C	21°C	24°C	28°C	21°C	24°C	28°C
N. vitripennis									
NV1	20.5±0.40 (29)*	16.1±0.20 (25)	12.0±0.20 (32)	0.33±0.04 (29)	0.36±0.05 (25)	0.40±0.06 (32)	7.2±7.0 (29)	6±2.8 (26)	2±1.0 (31)
NV2	25.1±0.46 (10)	17.7±0.37 (15)	14.1±0.46 (16)	0.15±0.08 (10)	0.10±0.03 (15)	0.10±0.02 (16)	51±12.0 (18)	34±11.8 (21)	29±8.0 (21)
N. giraulti									
NG1	26.2±01.08 (13)	18.0±0.39 (15)	14.2±0.26 (10)	0.15±0.06 (14)	0.25±0.09 (16)	0.26±0.16 (12)	69±14.8 (30)	0 (16)	77±12.4 (36)
NG2	24.6±.38 (24)	18.0±0.38 (20)	14.1±0.23 (15)	0.14±0.02 (25)	0.12±0.03 (20)	0.17±0.06 (16)	22±7.6 (29)	29±9.8 (25)	0 (16)
N. longicornis									
NL1	22.7±0.56 (21)	17.5±0.24 (29)	13.0±0.22 (24)	0.17±0.04 (20)	0.20±0.03 (29)	0.18±0.04 (26)	0 (22)	0 (29)	4 (26)
NL2	25.0±0.78 (15)	17.8±0.39 (17)	13.9±0.50 (15)	0.11±0.06 (15)	0.12±0.07 (16)	0.06±0.02 (14)	0 (15)	50±12.2 (28)	43±11.8 (24)

Table 3 Continued.

C

	Developmental Time				Proportion of males				Tendency to Diapause		
	21°C	24°C	28°C		21°C	24°C	28°C		21°C	24°C	28°C
N. vitripennis											
NV1	20.0+0.54 (30)*	16.2+0.42 (30)	12.0+0.17 (27)		0.37+0.04 (30)	0.34+0.06 (31)	0.37+0.06 (27)		5+5.0 (30)	3+2.8 (31)	3+3.6 (27)
NV2	22.7+0.24 (16)	17.7+0.30 (19)	14.2+0.49 (20)		0.12+0.02 (16)	0.15+0.04 (20)	0.14+0.03 (21)		32+8.6 (21)	19+4.8 (23)	26+5.4 (27)
N. giraulti											
NG1	24.0+0.20 (24)	17.9+0.42 (18)	14.3+0.35 (24)		0.18+0.04 (24)	0.18+0.06 (22)	0.20+0.04 (25)		25+6.2 (30)	15+6.8 (21)	17+7.6 (26)
NG2	19.7+.38 (12)	17.7+0.30 (10)	14.6+0.59 (9)		0.22+0.14 (12)	0.12+0.04 (14)	0.09+0.03 (10)		55+18.6 (20)	14+13.6 (10)	58+6.4 (23)
N. longicornis											
NL1	22.1+0.32 (28)	16.6+0.22 (36)	12.3+0.30 (27)		0.21+0.08 (28)	0.19+0.04 (36)	0.18+0.04 (28)		0 (28)	0 (36)	0 (28)
NL2	23.3+0.30 (15)	17.6+0.38 (18)	13.8+0.43 (16)		0.10+0.02 (15)	0.10+0.02 (18)	0.16+0.02 (17)		59+15.2 (27)	51+10.6 (28)	58+14.0 (28)

Table 4 Analysis of variance results for (a) developmental time, (b) proportion of males and (c) tendency to diapause for three species of *Nasonia* at 21° C, 24° C and 28° C.

Source	Type III Sums of Squares	df	Mean Square	F	P<
(a) Developmental time $R^2 = 0.999$					
Temperature	2920.733	2	1460.386	4303.920	0.001
Species	99.423	2	49.712	146.505	0.001
Strain(Species)	53.422	3	17.807	52.480	0.001
Temperature* Species	36.056	4	9.014	26.565	0.001
Temperature*Strain(Species)	23.801	6	3.967	11.691	0.001
Error	70.578	208	0.339		
(b) Sex Ratio $R^2 = 0.809$					
Temperature	0.169	2	8.449E-02	9.920	0.001
Species	0.282	2	0.141	16.564	0.001
Strain(Species)	0.688	3	0.229	26.935	0.001
Temperature*Species	5.622E-02	4	1.405E-02	1.650	NS*
Temperature*Strain(Species)	0.136	6	2.271E-02	2.666	0.05
Error	1.780	209	8.517E-03		
(c) Percent diapause $R^2 = 0.687$					
Temperature	1.732	2	0.866	9.686	0.001
Species	5.257	2	2.629	29.390	0.001
Strain(Species)	10.427	3	3.476	38.861	0.001
Temperature*Species	7.087	4	1.772	19.810	0.001
Temperature*Strain(Species)	2.843	6	0.474	5.298	0.001
Error	25.937	290	8.944E-02		

* NS indicates a non-significant factor

Table 5 Analysis of variance results for (a) developmental time, (b) sex ratio and (c) tendency to diapause for the three species of *Nasonia* with mothers and offspring being raised differentially across three temperatures. Mothers were reared at three temperatures (21 C 24 C and 28 C) and the larvae from each mother were reared at all three temperatures (see figure 1).

Source	Type III Sums of Squares	df	Mean Square	F	P<
(a) Developmental time R^2 = 0.998					
Maternal Temp	74.499	2	37.249	47.790	0.001
Larval Temp	14664.294	2	7332.147	9406.964	0.001
Species	267.865	2	133.932	171.832	0.001
Strain(Species)	395.323	3	131.744	169.024	0.001
Maternal Temp*Species	66.709	4	16.677	21.397	0.001
Maternal Temp*Strain(Species)	91.965	6	15.327	19.665	0.001
Maternal Temp*Larval Temp	108.281	4	27.070	34.730	0.001
Larval Temp*Species	58.996	4	14.749	18.923	0.001
Larval Temp*Strain(Species)	156.041	6	26.007	33.366	0.001
Maternal Temp*Larval Temp*Species	125.101	8	15.626	20.048	0.001
Maternal Temp*Larval Temp*Strain(Species)	101.022	12	8.419	10.801	0.001
Error	849.588	1090	0.779		
(b) Sex Ratio R^2 = 0.729					
Maternal Temp	0.606	2	0.303	18.222	0.001
Larval Temp	2.826E-02	2	1.413E-02	0.850	NS*
Species	1.202	2	0.601	36.132	0.001
Strain(Species)	5.071	3	1.696	101.624	0.001
Maternal Temp*Species	0.202	4	5.045E-02	3.033	NS
Maternal Temp*Strain(Species)	0.448	6	7.462E-02	4.486	0.001
Maternal Temp*Larval Temp	9.171E-02	4	2.293E-02	1.378	NS
Larval Temp*Species	1.436E-02	4	3.591E-03	0.216	NS
Larval Temp*Strain(Species)	0.130	6	2.164E-02	1.301	NS
Maternal Temp*Larval Temp*Species	0.193	8	2.411E-02	1.450	NS
Maternal Temp*Larval Temp*Strain(Species)	0.319	12	2.662E-02	1.600	NS
Error	18.596	1118	1.663E-02		
(c) Percent Diapause R^2 = 0.573					
Maternal Temp	0.987	2	0.494	5.459	0.05
Larval Temp	1.216	2	0.608	6.726	0.001
Species	3.638	2	1.819	20.122	0.001
Strain(Species)	35.523	3	11.841	130.969	0.001
Maternal Temp*Species	9.957	4	2.489	27.533	0.001
Maternal Temp*Strain(Species)	9.927	6	1.655	18.3	0.001
Maternal Temp*Larval Temp	0.421	4	0.105	1.163	NS
Larval Temp*Species	2.846	4	0.711	7.869	0.001
Larval Temp*Strain(Species)	1.114	6	0.186	2.053	NS
Maternal Temp*Larval Temp*Species	2.977	8	0.372	4.116	0.001
Maternal Temp*Larval Temp*Strain(Species)	7.956	12	0.663	7.333	0.001
Error	122.506	1355	9.042E-02		

* NS indicates a non-significant factor
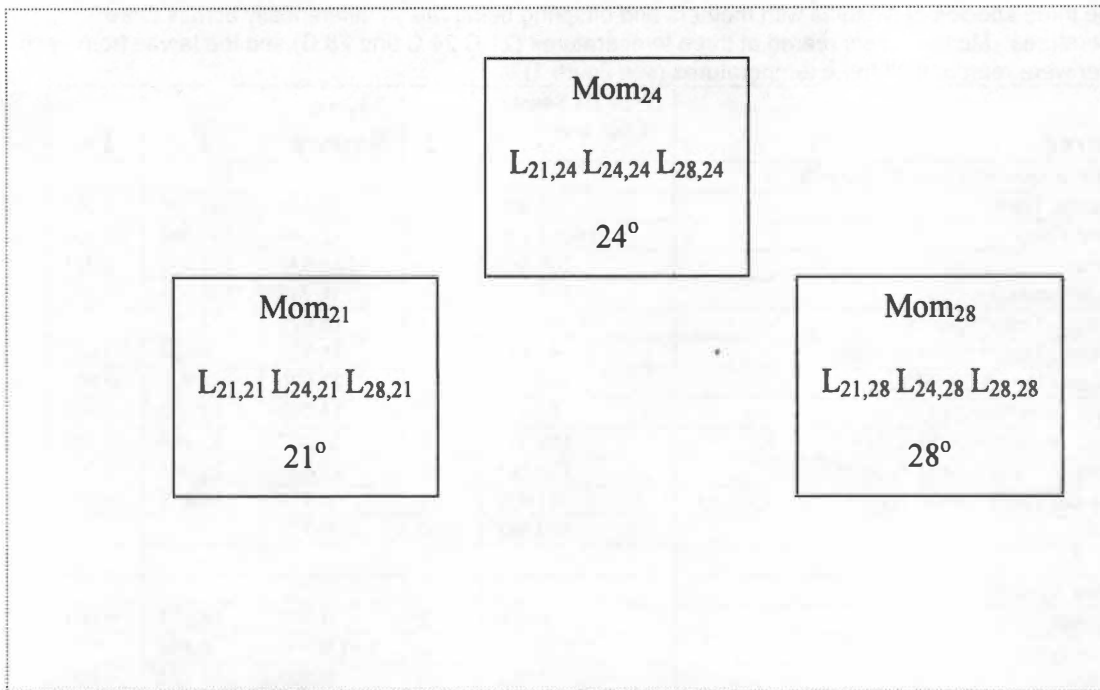
Figure 1 The maternal influence study design, mothers were raised in each of the three temperatures (21°, 24°, and 28° C), the clutches from each mother were split and the larvae were reared in each of three temperatures. The first subscript on the L (symbolizing a larval clutch) is the maternal temperature while the second is the larval temperature.

42

Figure 2

Reaction norms for (a) emergence time, (b) proportion of males and (c) tendency to diapause. Results from the preliminary study. See Figure 3 for legend definitions.
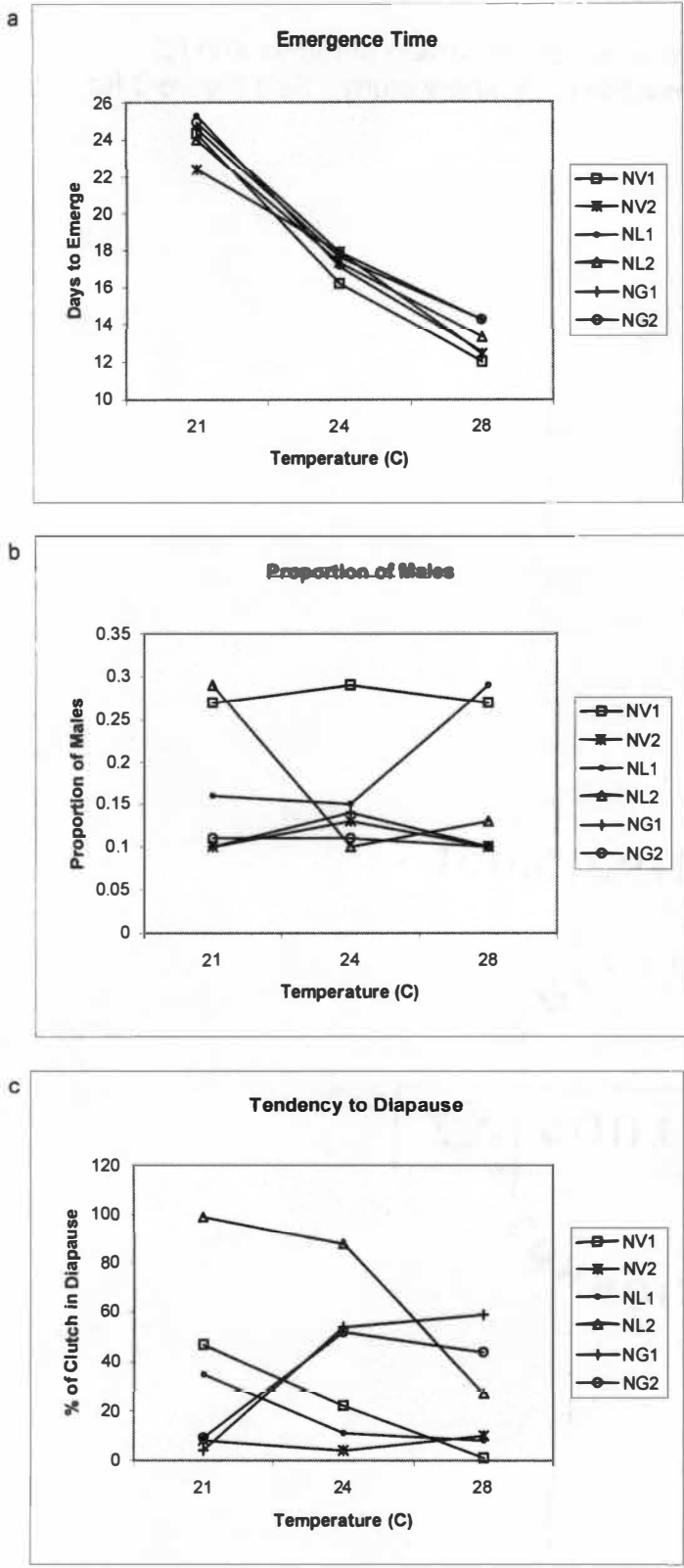
Figure 2

Figure 3
Reaction norms for emergence time for (a) all six strains and (b-d) strains separated by species, means ± 2 standard errors are shown across temperatures. NV1 = NVOH, NV2 = NVXNVB, NL1 = NLCA, NL2 = NLUT, NG1 = NGPA and NG2 = RV2.
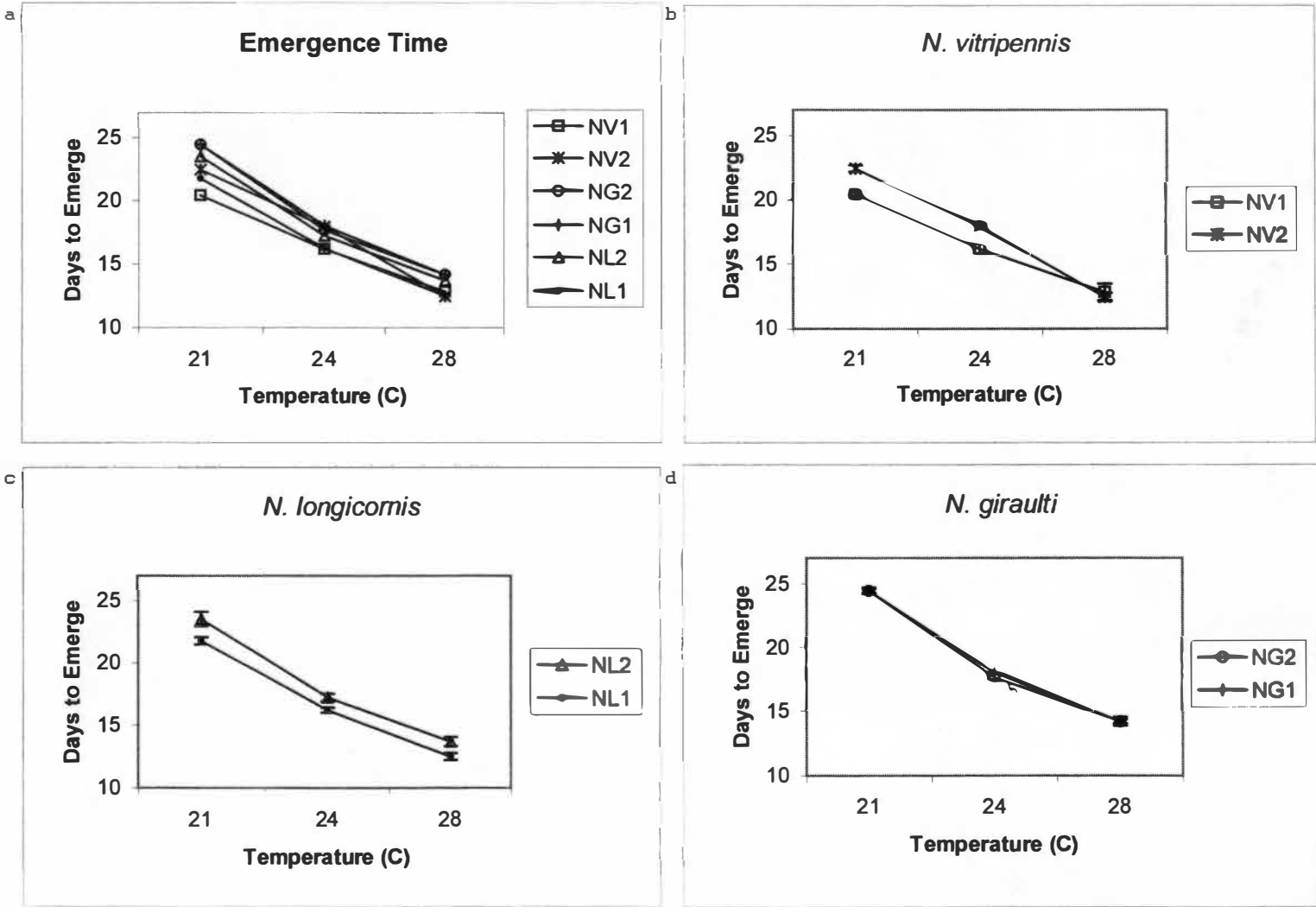
Figure 3

Figure 4
Reaction norms for proportion of males for (a) all six strains and (b-d) strains separated by species, means ± 2 standard errors are shown across temperatures. See Fig 2 for legend definitions.
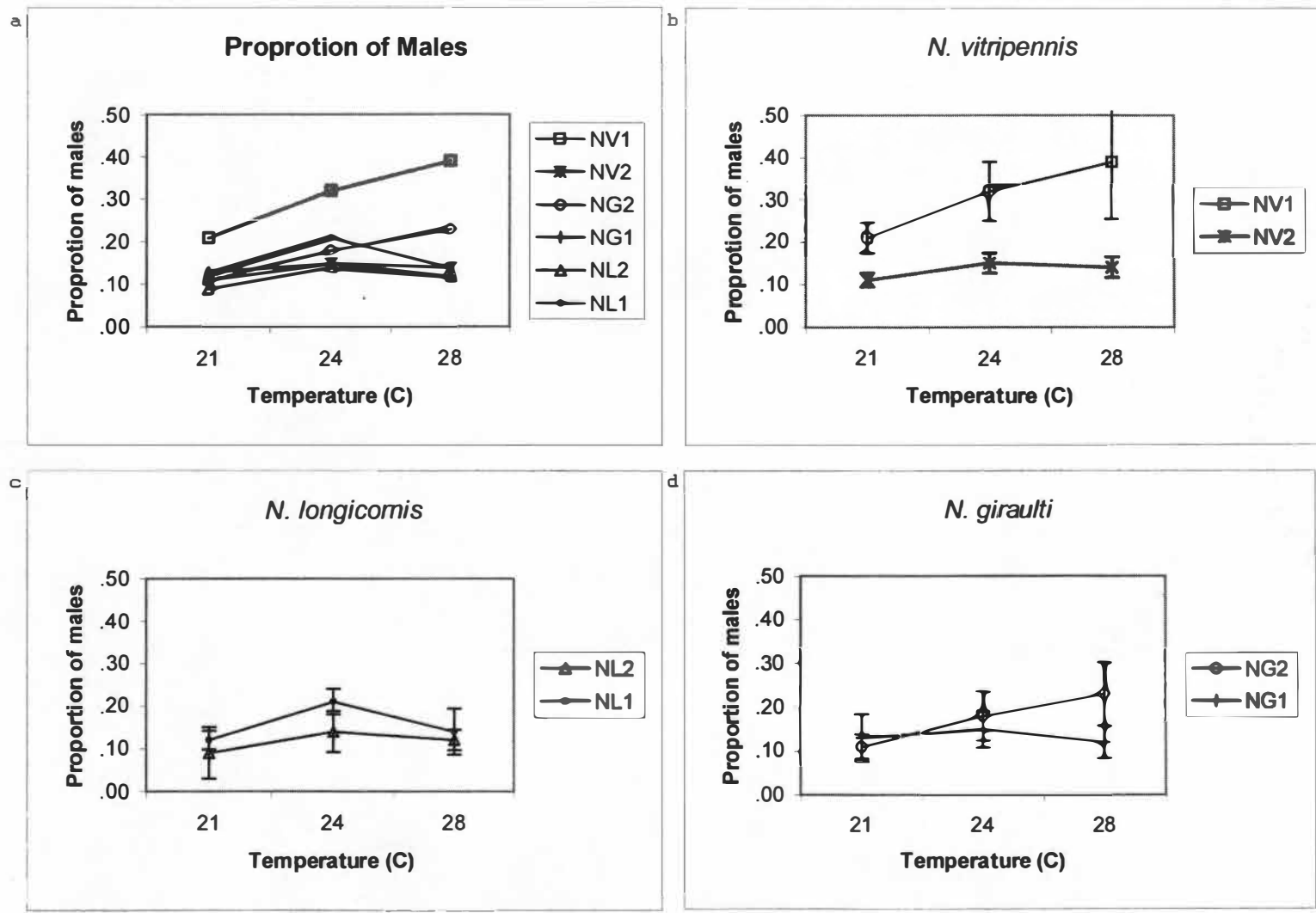
Figure 4

Figure 5
Reaction norms for the tendency to diapause for (a) all six strains and (b-d) strains separated by species, means ± 2 standard errors are shown across temperatures. See Fig 2 for legend definitions.
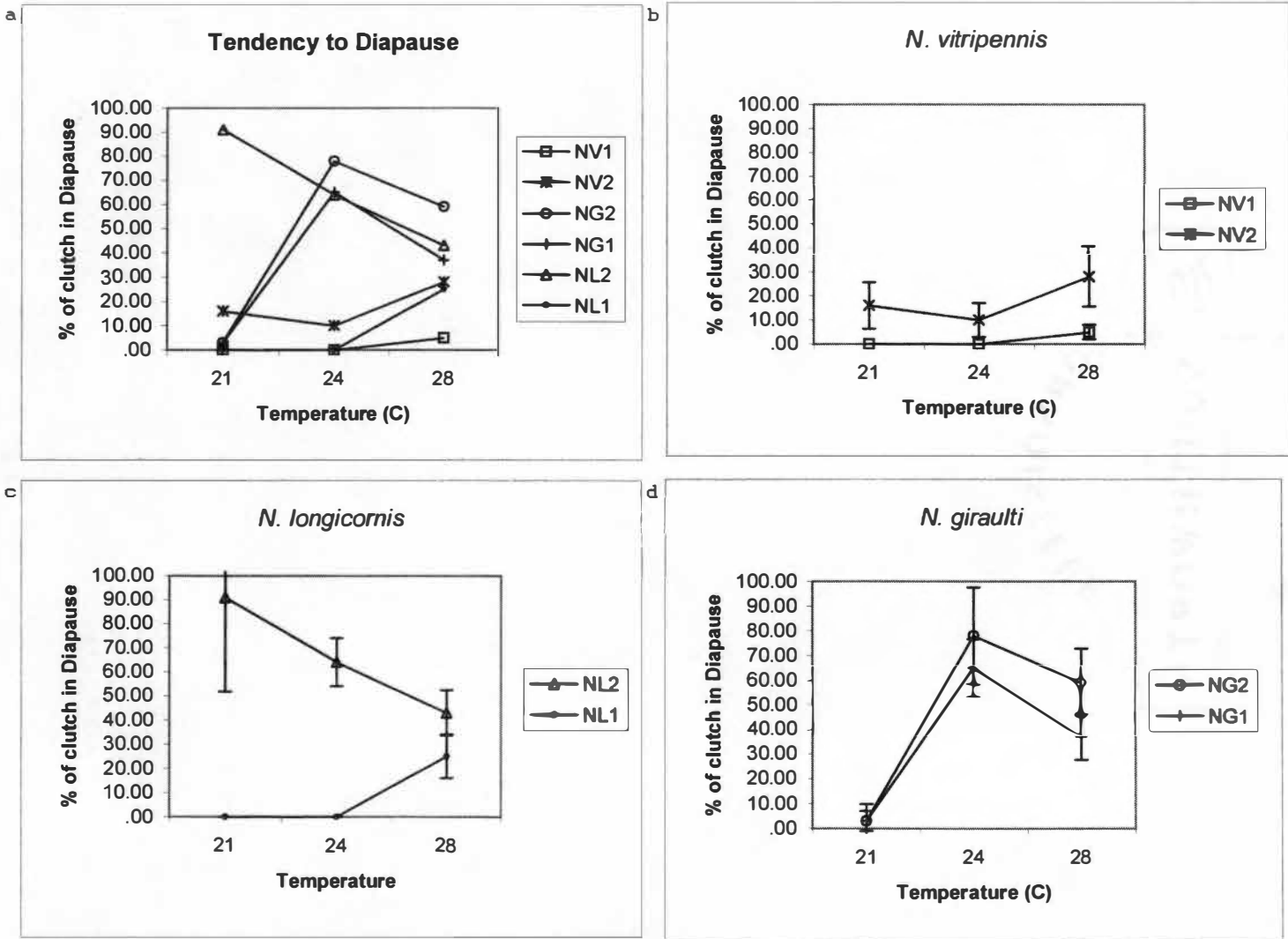
Figure 5

Figure 6
Reaction norms for (a) emergence time, (b) proportion of males and (c) tendency to diapause across temperatures for the three species of *Nasonia*. These are summaries of the data in Figs 2-4. NV = *N. vitripennis*, NL = *N. longicornis* and NG = *N. giraulti.*
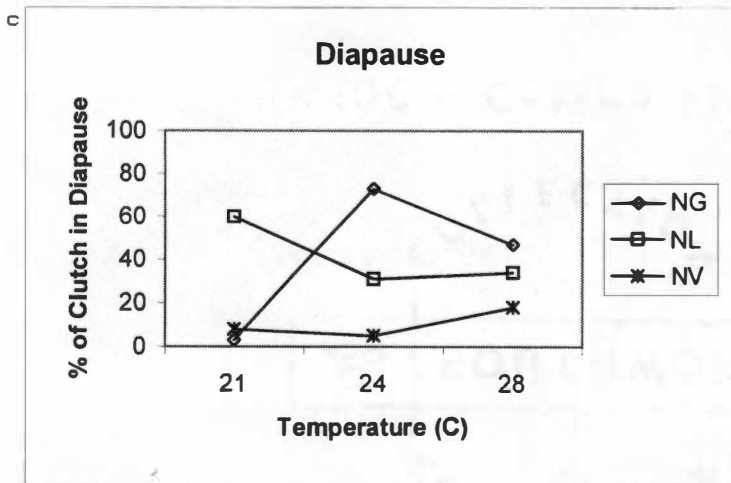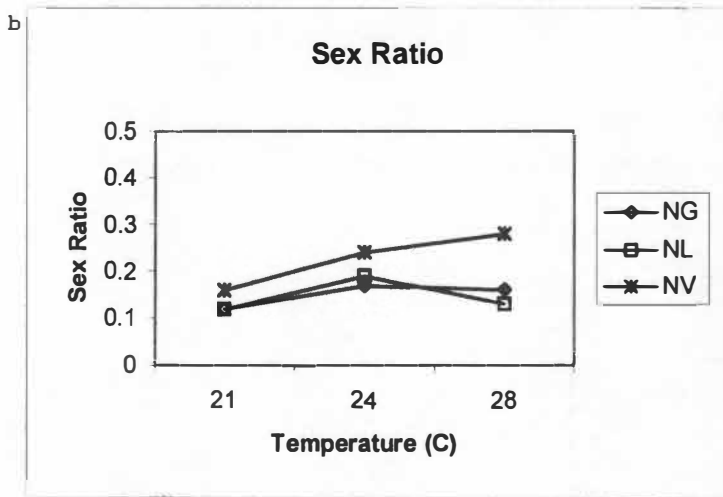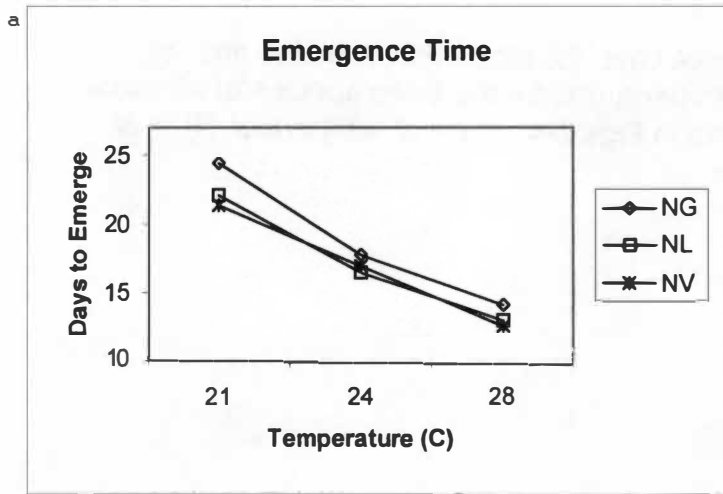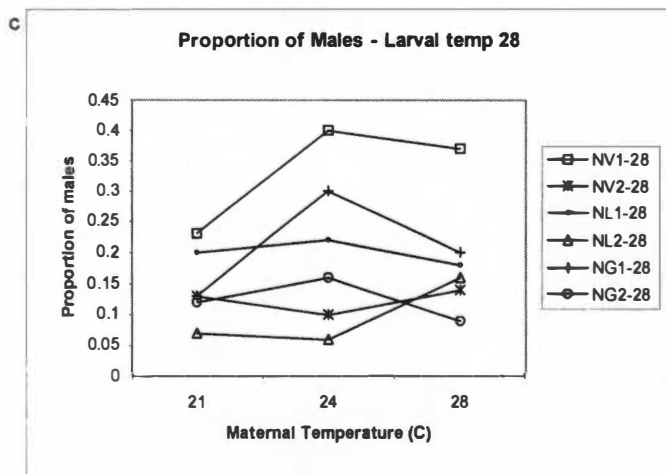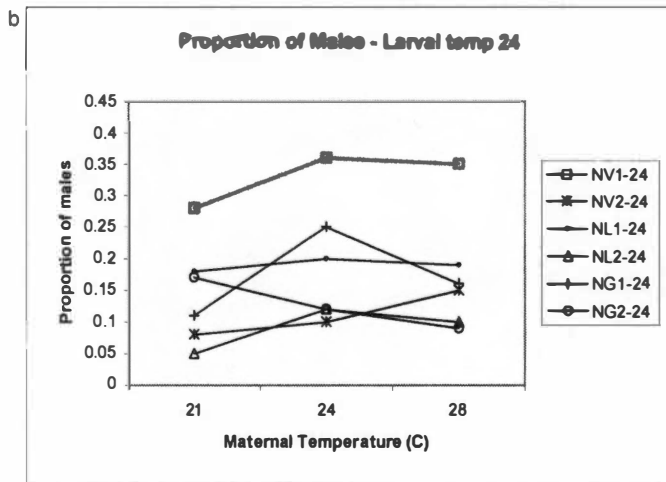
Figure 6

Figure 7
Reaction norm plots for emergence time for (a) maternal temperature 21° C (b) maternal temperature 24° C and (c) maternal temperature 28° C. All are across the three larval temperatures. See Figure 3 for legend definitions.
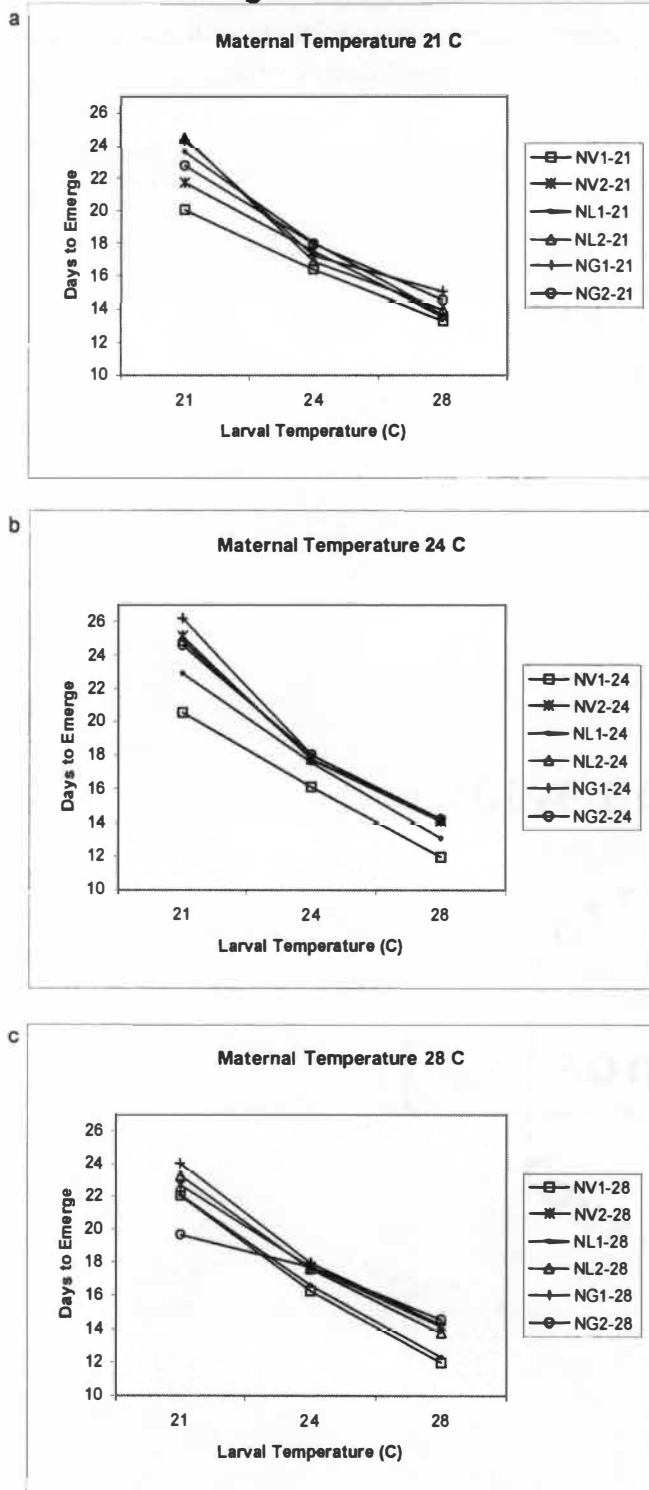
Emergence Time

Figure 7

Figure 8
Reaction norm plots for emergence time for (a) larval temperature at 21° C (b) larval temperature 24° C and (c) larval temperature 28° C. All are across the three maternal temperatures. See Figure 3 for legend definitions.

Emergence Time
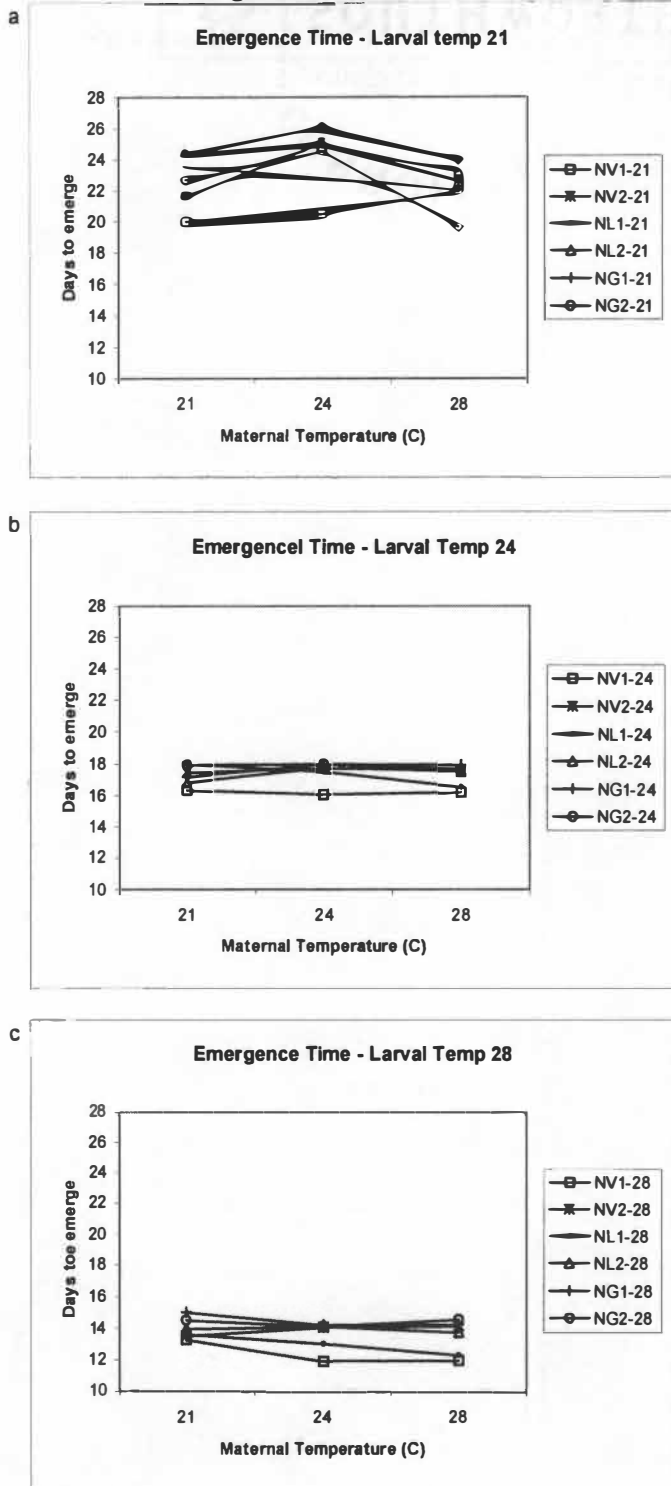
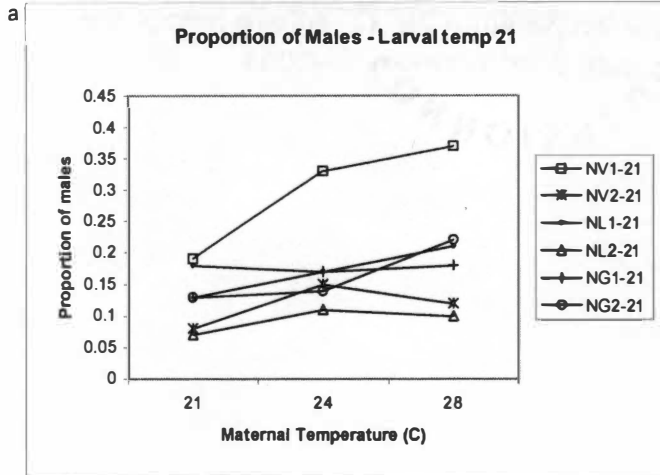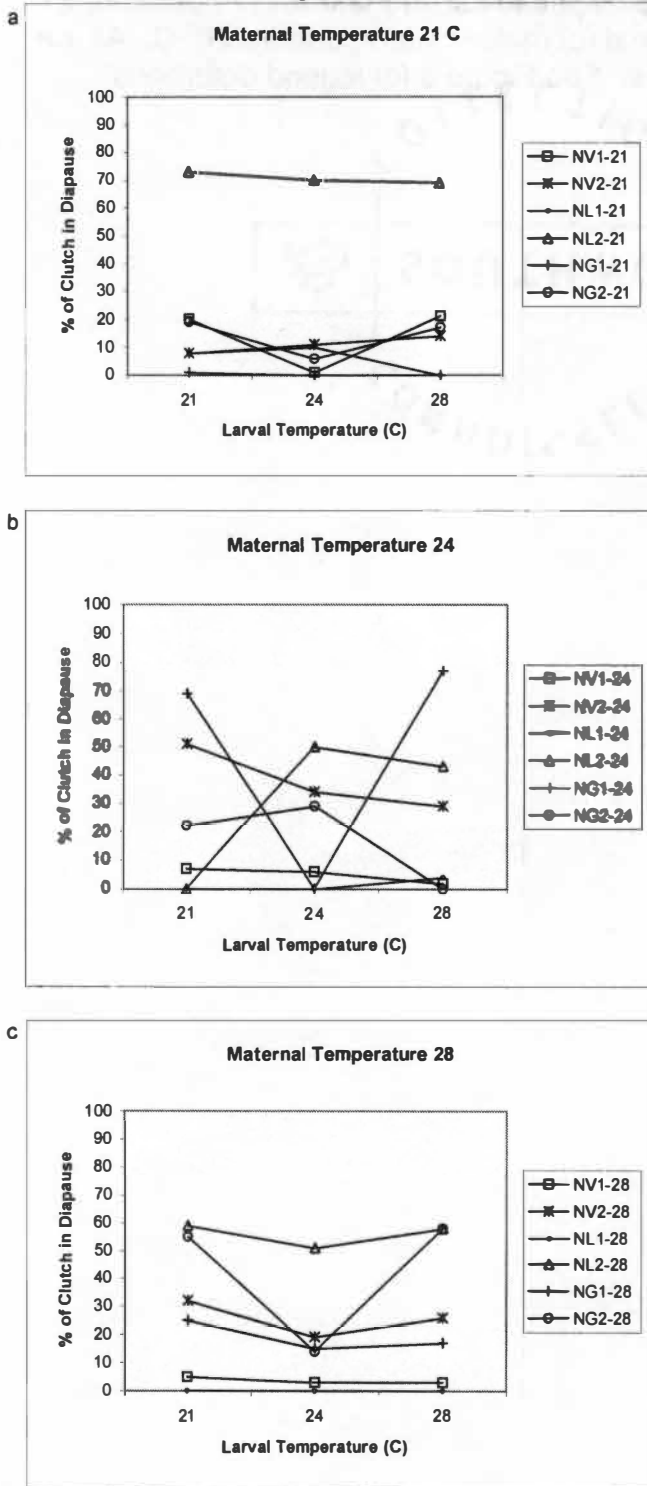Figure 8

Figure 9
Reaction norm plots for proportion of males for (a) larval temperature 21° C (b) larval temperature 24° C and (c) larval temperature 28° C. All are across the three maternal temperatures. See Figure 3 for legend definitions.

Proportion of Males

Figure 9

Figure 10
Reaction norm plots for tendency to diapause for (a) maternal temperature 21° C (b) maternal temperature 24° C and (c) maternal temperature 28° C. All are across the three larval temperatures. See Figure 3 for legend definitions.
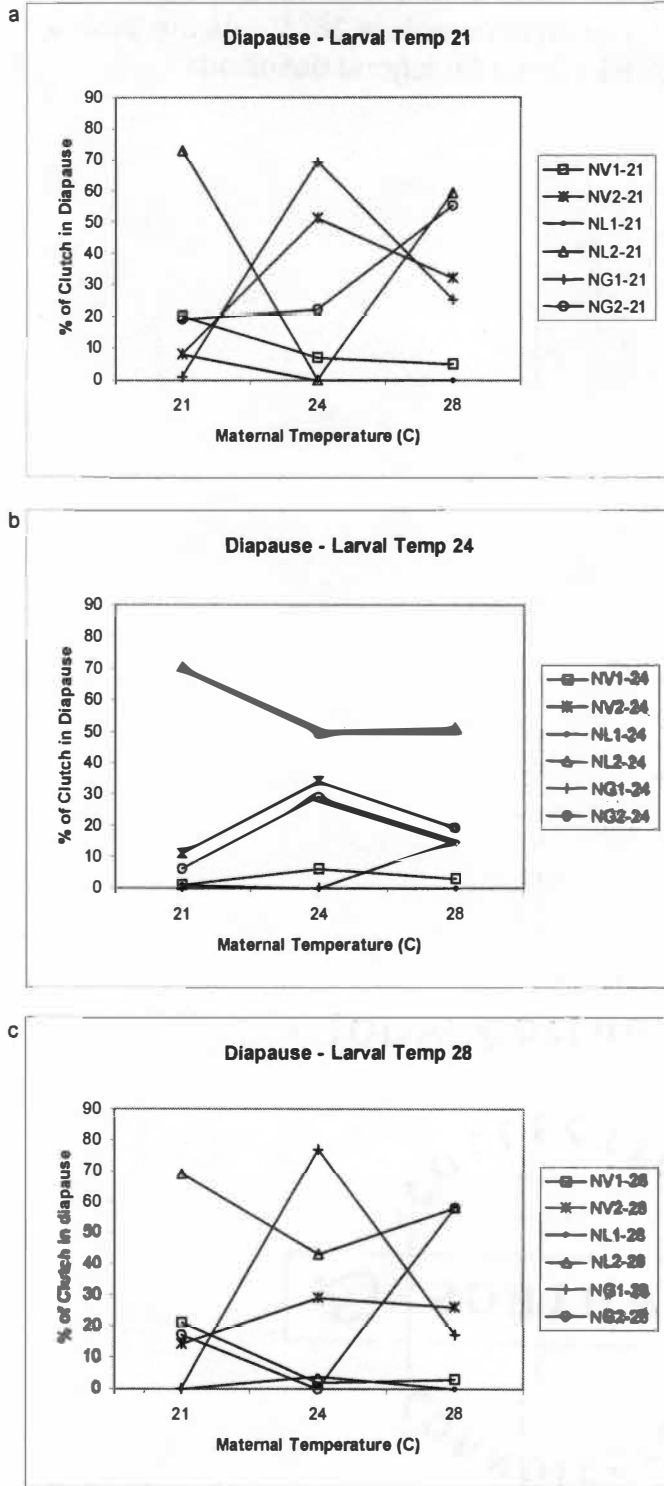
Tendency to Diapause

Figure 10

Figure 11
Reaction norm plots for the tendency to diapause for (a) larval temperature 21° C (b) larval temperature 24° C and (c) larval temperature 28° C. All are across the three maternal temperatures. See Figure 3 for legend definitions.

Tendency to Diapause

a

Diapause - Larval Temp 21

b

Diapause - Larval Temp 24

c

Diapause - Larval Temp 28

Figure 11

Figure 12
Reaction norms for (a) emergence time, (b) proportion of males and (c) tendency to diapause. Results from maternal influence study, data from when larval temperature was the same as the maternal temperature. See Figure 3 for legend definitions.
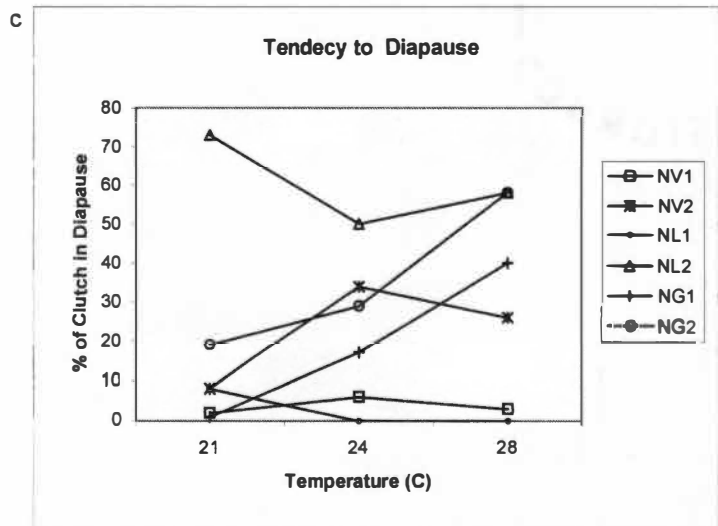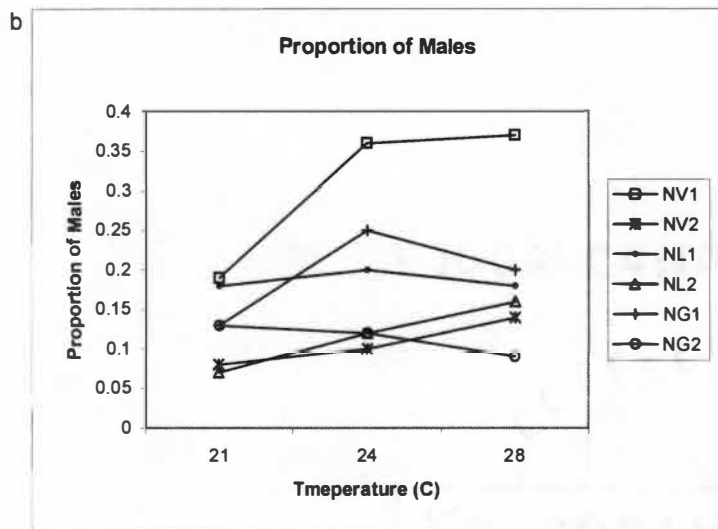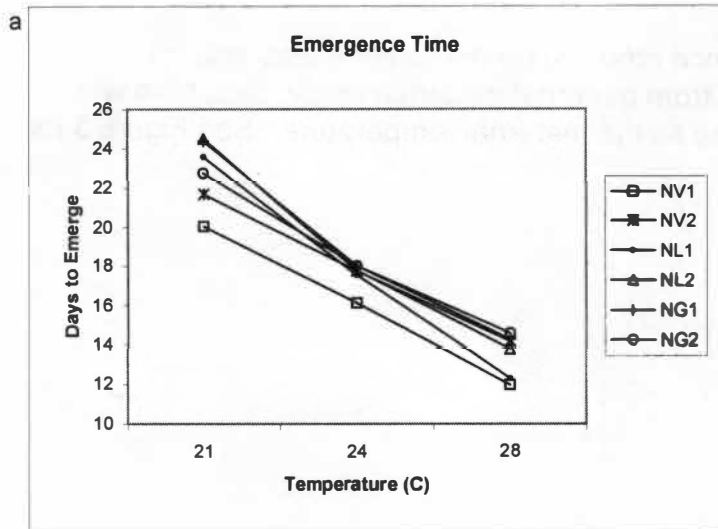
Figure 12

64

Vita

Anne Grinnnan got her Bachelor of Arts in Biology at Augustana College in Rock Island, Illinois in May 2000. After that she continued directly on to University of Tennessee where she will graduate with a Master of Science degree in Ecology and Evolution in August 2003. Next, she plans on taking a break from attending school and working in an education program.