Chapter 4

Life history traits in populations of *Tetrastichus coeruleus* (Hymenoptera: Eulophidae) that differ in ecology, *Wolbachia* infection frequency and mode of reproduction

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Life history strategies are characterized by trade-offs, in particular between investment in reproduction and survival. Differences in life history traits between populations may reflect adaptive strategies that optimize reproduction and survival in relation to their respective environments. Furthermore, life history strategies may vary with the mode of reproduction or may be manipulated by parasites. Quantifying differences in life history traits between populations that differ in these aspects is an important first step in identifying the roles of these factors in shaping life history variation.

The parasitoid wasp *Tetrastichus coeruleus* occurs both in natural and agricultural environments. Populations in natural areas are infected with parthenogenesis-inducing *Wolbachia* bacteria and reproduce asexually, whereas populations from agricultural fields are not infected and reproduce sexually.

In this paper, we describe differences in life history traits between two populations of *T. coeruleus*, one from a natural (Meijendel) and one from an agricultural (Brabant) environment. We quantified clutch size, life span, female weight and female nutrient concentrations (proteins, lipids, sugars and glycogen).

Females from Brabant laid larger clutches, had longer life spans and were heavier than females from Meijendel. There was no difference in the relative amounts of proteins, lipids or sugars, but females from Meijendel had relatively more glycogen

than females from Brabant.

The two populations therefore exhibit markedly different life history strategies. Females from Brabant invest relatively more in survival, body size and clutch size, whereas females from Meijendel seem to be more active or fly longer distances. Further studies are needed to determine if and how these life history differences are related to differences in ecology, mode of reproduction or *Wolbachia* infection.

Introduction

Life history traits are involved in the timing and duration of key events in an organism's life, in particular reproduction and survival. Important life history traits are development time, (reproductive) life span, number and size of offspring, adult size and mortality rate (Roff 1992, 2002, Stearns 1992). In order to maximize fitness, an organism needs to optimize each of these parameters. However, as resources can be used only once, it is not possible to maximize reproduction and survival at the same time. Therefore, life history traits are subject to trade-offs, such as between longevity and fecundity or between number and size of offspring (Roff 1992, 2002, Stearns 1992). The allocation of resources is thus an important aspect in life history trade-offs.

There are three essential nutrient resources: proteins, lipids and carbohydrates. In parasitoid wasps, proteins are mainly used for egg production, but also for maintenance, carbohydrates are mainly used for maintenance and lipids can be used for both (Jervis & Kidd 1986, Heimpel & Collier 1996, Rivero & Casas 1999). For example, in the parasitoid wasp *Asobara tabida* lipids are used for metabolic maintenance (survival), flight and egg production (Ellers 1996, Ellers & van Alphen 1997, Ellers *et al.* 1998). Of these three nutrients, proteins can be obtained through host feeding as an adult or carried over from the host during the larval stage. Carbohydrates can be obtained from sugar meals, such as nectar or honey, and are stored as glycogen. Lipids, stored as triglycerides, provide more energy per unit weight and take up less storage space than carbohydrates (Rivero & Casas 1999, Visser & Ellers 2008). However, since most parasitoid wasps cannot biosynthesize lipids (Visser *et al.* 2010), lipid reserves need to be carried over from the host during the larval stage. Therefore, carbohydrates provide a first, short-term energy supply, whereas lipids provide a long-term energy supply and are used when carbohydrate reserves are low (Rivero & Casas 1999, Visser & Ellers 2008).

Differences in life history strategies between populations may result from differences in ecology, mode of reproduction or from manipulation by parasites (Roff 1992, 2002, Stearns 1992). Differences in ecology between populations are probably the most obvious cause of differences in life history strategies. For example, the parasitoid wasp *Leptopilina boulardi* exhibits differences in egg load, life span, activity and energy reserves (amount of lipids, sugars and glycogen) between populations that differ in climatic factors and host distribution (Moiroux *et al.* 2010, Seyahooei

2010). The parasitoid wasp *Asobara tabida* exhibits differences in virulence against its host, diapause, survival, adult size, egg load and fat reserves, which are caused by differences in food- and host-availability between geographically different populations (Kraaijeveld & van der Wel 1994, Kraaijeveld & van Alphen 1994, 1995, Ellers & van Alphen 1997, Kraaijeveld & Godfray 1999).

However, different modes of reproduction may also result in different life history strategies. Individuals from sexual populations are less closely related to each other than individuals from asexual populations (e.g. chapter 3, Reumer *et al.* in prep). The level of competition for resources between individuals may therefore be different between sexual and asexual populations.

Alternatively, the presence or absence of a microbial infection may also cause differences in life history traits (Gross *et al.* 2009). For example, the parasitoid wasp *Leptopilina boulardi* is infected with a viral symbiont that manipulates the superparasitism behaviour of its host in order to enhance its own horizontal transmission (Varaldi *et al.* 2003). *Wolbachia* bacteria also have been shown to affect life history traits in several of its hosts. For example, *Wolbachia*-infected *Drosophila melanogaster* females had a longer life span and increased competitiveness compared to uninfected females (Alexandrov *et al.* 2007), *Wolbachia* had a positive effect on fecundity of *D. melanogaster* during periods of nutritional stress (Brownlie *et al.* 2009) and female longevity and fecundity of the psocid *Liposcelis tricolor* decreased dramatically when they were cured from their *Wolbachia* infection (Jia *et al.* 2009). Moreover, the parasitoid wasp *Asobara tabida* is dependent on *Wolbachia* for completing oogenesis (Dedeine *et al.* 2001, Kremer *et al.* 2010).

The parasitoid wasp *Tetrastichus coeruleus* (Hymenoptera: Eulophidae) is a gregarious, synovigenic, egg-larval parasitoid of the common asparagus beetle (*Crioceris asparagi*). *T. coeruleus* both feeds on and parasitizes the eggs of *C. asparagi* (Capinera & Lilly 1975a, van Alphen 1980). *C. asparagi* lives on the asparagus plant (*Asparagus officinalis*), which grows on sandy soils, such as coastal dune areas, and as a crop in monoculture on agricultural fields. Interestingly, populations of *T. coeruleus* from the Dutch coastal dune areas are highly female-biased (more than 92% females), while populations from the Dutch agricultural fields have nearly equal sex ratios (55% females) (chapter 2, Reumer *et al.* 2010). The populations from the Dutch coastal dune areas are infected with *Wolbachia* bacteria that induce parthenogenesis, whereas populations from the Dutch agricultural fields are not infected and reproduce sexually (chapter 2, Reumer *et al.* 2010).

In this paper, we compare life history traits between two populations of *T. coeruleus* that differ in ecology, *Wolbachia* infection and mode of reproduction as part of a larger study into the interplay between these three factors. We measured clutch sizes (the number of emerged wasps per host pupa), life span, female weight and the amounts of four nutrient resources (proteins, lipids, sugars and glycogen) in females at the time of emergence from the host pupa. We discuss possible explana-

tions for differences in life history traits between the two populations.

Materials and Methods

Tetrastichus coeruleus populations

Two populations of the parasitoid wasp *Tetrastichus coeruleus* (Hymenoptera: Eulophidae) were used in all experiments. One population was sampled from agricultural fields in Brabant (BR), The Netherlands. This population was not infected with *Wolbachia* and reproduced sexually (chapter 2, Reumer *et al.* 2010). Both males and females were collected from this population (ca. 43% males; chapter 2, Reumer *et al.* 2010). The other population was sampled from the natural dune area Meijendel (MD), The Netherlands. This population was infected with *Wolbachia* and reproduced parthenogenetically (chapter 2, Reumer *et al.* 2010). However, a small percentage of males was produced in this population (ca. 7% males; chapter 2, Reumer *et al.* 2010). More details about the sampled locations can be found in chapter 2, Reumer *et al.* (2010). In the spring and summer of 2009 and 2010 individuals from both populations were obtained from the field by collecting larvae of the asparagus beetle *Crioceris asparagi*, the host of *T. coeruleus*. These larvae were reared in the lab at 20°C, light:dark 16:8, 65% relative humidity. Some of these had been parasitized by *T. coeruleus*, in which case wasps instead of beetles would emerge from the pupae. The sex of each wasp was determined by ascertaining under a binocular the presence of a groove on the ventral side of the abdomen which envelopes the ovipositor. In males this groove is absent.

Clutch size

To test for differences in clutch size between pupae from Brabant and Meijendel, we scored how many wasps emerged from each host pupa (collection years 2009 and 2010, which were also used for the life span and physiology experiments, respectively).

Life span

To test for differences in life span between individuals from Brabant and Meijendel, we measured the life span of all males and females that emerged from the host pupae (collection year 2009). After emergence, the wasps were transferred to a glass tube (2.5 x 8.0 cm) with a medium of agar and a foam stopper with a drop of honey and kept individually at 20°C, light:dark 16:8, 65% relative humidity. Deaths were recorded daily, until all individuals were dead.

Physiology

To test for physiological differences between females from Brabant and Meijendel, we used unrelated zero-to-one-day-old virgin females (collection year 2010), which were stored individually in a 1.5 ml Eppendorf tube and kept at -80°C until mea-

surement. During measurement, samples were kept on ice at all times. Each female was weighted to the nearest µg before other measurements were taken. The measured amounts of proteins, lipids, sugars and glycogen were corrected for the total weight of the female.

The amounts of proteins, lipids, sugars and glycogen were quantified following the method described by Van Handel (1985a, 1985b) and Van Handel & Day (1988) and modified by Giron *et al.* (2002). Individual females were crushed with a plastic pestle in 300 µl methanol and centrifugated at 180 g for 15 minutes.

For the protein analysis, 100 µl of the supernatant was transferred to a new 1.5 ml Eppendorf tube and 700 µl physiological water and 200 µl filtered Bradford reagent (Bio-Rad, Hercules, CA, USA) were added. After 45 minutes at room temperature, the absorbance was measured at 595 nm using an Ultrospec II spectrophotometer (Pharmacia LKB, Uppsala, Sweden).

We then added 100 µl methanol, 150 µl chloroform and 60 µl 2% sodium sulphate to the original sample (200 µl supernatant and precipitate). After centrifugation at 180 g for 15 minutes, lipids and sugars remain dissolved in the supernatant, while the precipitate contains the glycogen. The supernatant was transferred to a new 1.5 ml Eppendorf tube and both the supernatant and precipitate were stored on ice.

For the lipid analysis, 50 µl of the supernatant was transferred to a new 1.5 ml Eppendorf tube and heated at 90°C until near complete evaporation. Then, 40 µl 98% sulphuric acid was added and the sample was reheated at 90°C for two minutes and cooled on ice for 5 minutes, after which 960 µl vanillin reagent was added. After 20 minutes at room temperature, the absorbance was measured at 525 nm using a spectrophotometer.

For the sugar analysis, 80 µl of the supernatant was transferred to a new 1.5 ml Eppendorf tube and heated at 90°C until near complete evaporation. Then, 1 ml anthrone reagent was added and the sample was left at room temperature for 15 minutes, after which it was reheated at 90°C for 15 minutes. After cooling on ice for 5 minutes, the absorbance was measured at 625 nm using a spectrophotometer.

For the glycogen analysis, the precipitate was washed twice by adding 400 µl 80% methanol and centrifugating it at 180 g for 5 minutes, after which the supernatant was removed. Then, 1 ml anthrone reagent was added and the sample was left at room temperature for 15 minutes, after which it was reheated at 90°C for 15 minutes. After cooling on ice for 5 minutes, the absorbance was measured at 625 nm using a spectrophotometer.

A fresh control sample and a blank sample were measured during each round of absorbance measurements. Amounts of proteins, lipids, sugars and glycogen were calculated using standard calibration curves of known concentrations of bovine serum albumin for proteins, sunflower oil for lipids and glucose for sugars and glycogen.

Statistical analysis

Statistical analyses were performed in R (version 2.13.1; R Developmental Core Team, 2011).

Analyses of variance (anova) were used to test for differences in clutch size between pupae from Brabant and Meijendel. Significance of explanatory variables (population and collection year) was tested by dropping (interactions between) explanatory variables from the model and comparing the resulting change in variance using an F-test. Clutch size means and their standard errors are reported.

Survival analyses (in the 'survival' package) were used to test for differences in life span between individuals from Brabant and Meijendel and between females and males within populations. Significance of explanatory variables (population, sex and clutch size) was examined in a cox proportional hazard model using a score log rank test. Significance of interactions between explanatory variables was tested by dropping the interactions from the model and comparing the resulting change in deviance to a χ^2-distribution. Life span means and their standard errors are reported.

Analyses of covariance (ancova) were used to test for differences in weight and amounts of proteins, lipids, sugars and glycogen between females from Brabant and Meijendel. Significance of explanatory variables (population, clutch size and female weight) was tested by dropping (interactions between) explanatory variables from the model and comparing the resulting change in variance using an F-test. Means and their standard errors are reported.

Results

Clutch size

We scored the clutch sizes of 683 parasitized host pupae: 551 pupae from Brabant (330 collected in 2009 and 221 in 2010) and 132 pupae from Meijendel (43 collected in 2009 and 89 in 2010).

Clutch sizes in Brabant ranged between 1 and 22 wasps per pupa in 2009, with 7 wasps per pupa as the most frequently observed clutch size (Fig. 4.1), while in 2010 clutch sizes ranged between 1 and 19 wasps per pupa, with 6 wasps per pupa as the most frequently observed clutch size (Fig. 4.2). Clutch sizes in Meijendel ranged between 1 and 13 wasps per pupa in 2009, with 2 and 8 wasps per pupa as the most frequently observed clutch sizes (Fig. 4.1), while in 2010 clutch sizes ranged between 1 and 9 wasps per pupa, with 6 wasps per pupa as the most frequently observed clutch size (Fig. 4.2).

Overall, pupae from Brabant had a significantly larger clutch size than pupae from Meijendel (mean clutch size = 6.64 ± 0.15 and 4.79 ± 0.22 wasps per pupa, respectively) and the clutch sizes from pupae collected in 2009 were significantly larger than the clutch sizes from pupae collected in 2010 (mean clutch size = 6.77 ± 0.18 and 5.70 ± 0.19 wasps per pupa, respectively) (overall: $F_{3,679} = 14.45$, $p <$

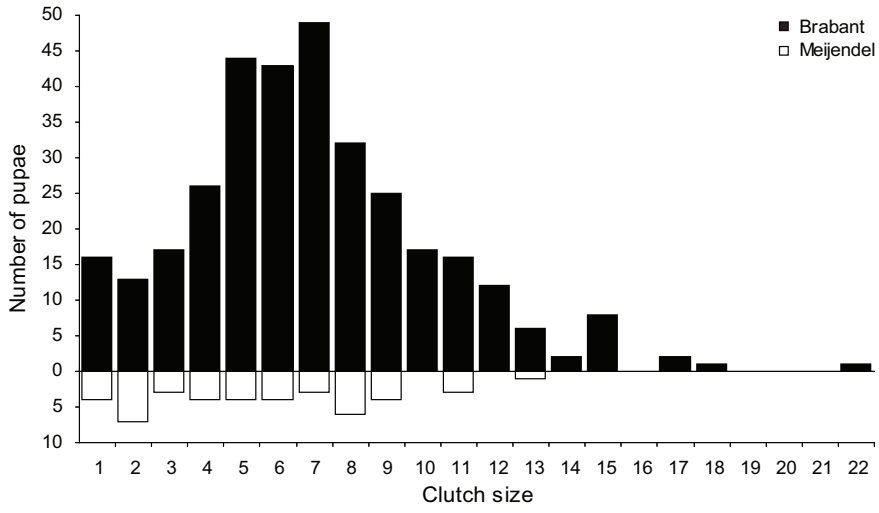

Figure 4.1: Frequency of clutch sizes (number of wasps per host pupa) of *Tetrastichus coeruleus* for pupae from Brabant and Meijendel in collection year 2009.

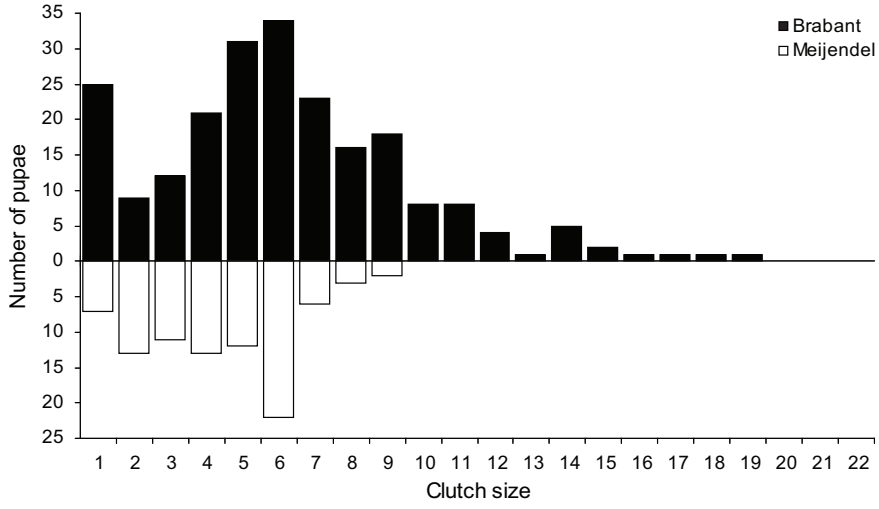

Figure 4.2: Frequency of clutch sizes (number of wasps per host pupa) of *Tetrastichus coeruleus* for pupae from Brabant and Meijendel in collection year 2010.

0.0001; population: $F_{1,681} = 33.28$, $p < 0.0001$; year: $F_{1,681} = 17.45$, $p < 0.0001$). There was no significant effect of the interaction between population and collection year ($F_{1,679} = 0.31$, $p = 0.58$).

Both within 2009 and 2010, pupae from Brabant had a significantly larger clutch size than pupae from Meijendel (mean clutch size 2009 = 6.94 ± 0.19 and 5.53 ± 0.49 wasps per pupa, respectively; $F_{1,371} = 6.49$, $p = 0.01$; Fig. 4.1; mean clutch size 2010 = 6.21 ± 0.24 and 4.43 ± 0.21 wasps per pupa, respectively; $F_{1,308} = 19.81$, $p < 0.0001$; Fig. 4.2).

Also, both within Brabant and Meijendel, the clutch sizes from pupae collected in 2009 were significantly larger than the clutch sizes from pupae collected in 2010 (mean clutch size Brabant = 6.94 ± 0.19 and 6.21 ± 0.24 wasps per pupa, respectively; $F_{1,549} = 5.83$, $p = 0.02$; mean clutch size Meijendel = 5.53 ± 0.49 and 4.43 ± 0.21 wasps per pupa, respectively; $F_{1,130} = 5.79$, $p = 0.02$).

Life span

We measured the life span of 2424 individuals: 1270 females and 926 males from Brabant and 217 females and 11 males from Meijendel.

Females from Brabant had significantly longer life spans than females from Meijendel (Fig. 4.3; mean survival = 33.03 ± 0.31 and 29.24 ± 0.65 days, respectively; overall: $Z = 31.86$, $df = 3$, $p < 0.0001$; population: $Z = 31.21$, $df = 1$, $p < 0.0001$). There was no significant effect of clutch size or the interaction between population and clutch size on female life span (clutch size: $Z = 1.02$, $df = 1$, $p = 0.31$; interaction: $\chi^2 = 0.09$, $df = 1$, $p = 0.77$).

There was no significant difference in life span between males from Brabant and Meijendel (mean survival = 30.69 ± 0.37 and 32.45 ± 2.89 days, respectively; overall: $Z = 3.81$, $df = 3$, $p = 0.28$; population: $Z = 0.01$, $df = 1$, $p = 0.92$). Also, there was no significant effect of clutch size or the interaction between population and clutch size on male life span (clutch size: $Z = 3.79$, $df = 1$, $p = 0.052$; interaction: $\chi^2 = 0.02$, $df = 1$, $p = 0.88$).

Within Brabant, females had significantly longer life spans than males (Fig. 4.4; mean survival = 33.03 ± 0.31 and 30.69 ± 0.37 days, respectively; overall: $Z = 18.56$, $df = 3$, $p = 0.0003$; sex: $Z = 13.97$, $df = 1$, $p = 0.0002$). There was no significant effect of clutch size or the interaction between sex and clutch size on life span (clutch size: $Z = 2.03$, $df = 1$, $p = 0.15$; interaction: $\chi^2 = 3.20$, $df = 1$, $p = 0.07$).

Within Meijendel, there was no significant difference in life span between females and males (mean survival = 29.24 ± 0.65 and 32.45 ± 2.89 days, respectively; overall: $Z = 0.79$, $df = 3$, $p = 0.85$; sex: $Z = 0.72$, $df = 1$, $p = 0.39$). Also, there was no significant effect of clutch size or the interaction between sex and clutch size on life span (clutch size: $Z = 0.05$, $df = 1$, $p = 0.82$; interaction: $\chi^2 = 0.001$, $df = 1$, $p = 0.97$).

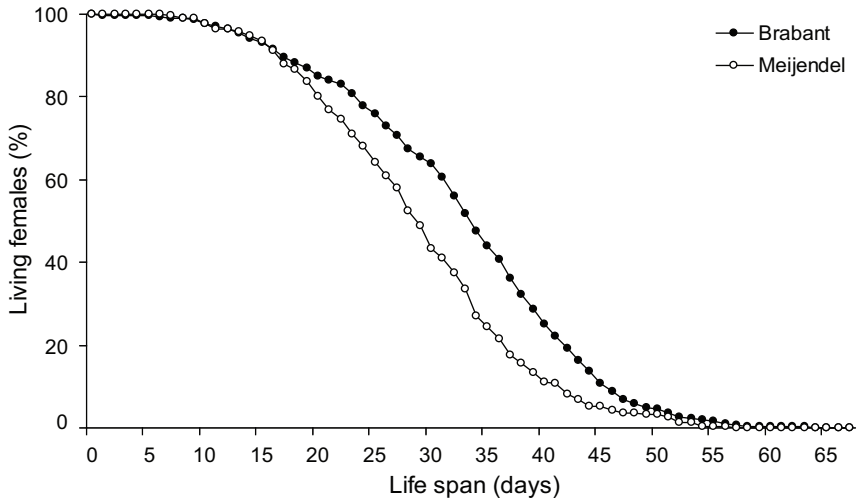

Figure 4.3: Survival probability of *Tetrastichus coeruleus* females from Brabant (black dots) and Meijendel (white dots).

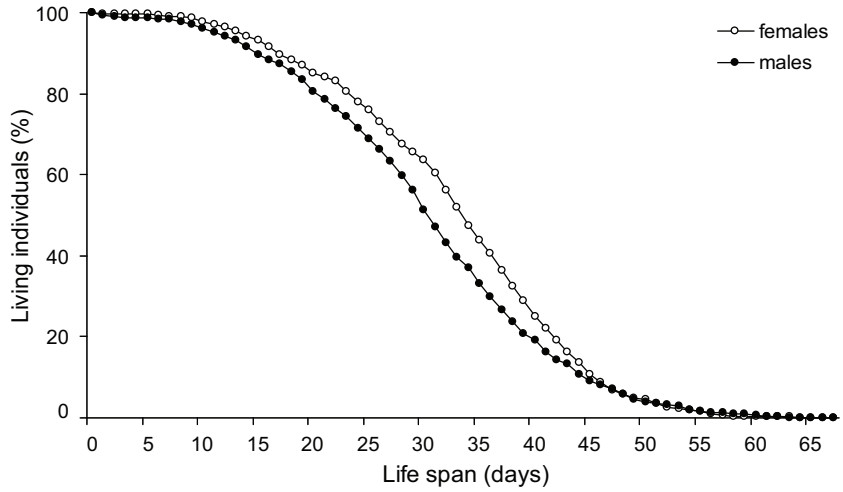

Figure 4.4: Survival probability of *Tetrastichus coeruleus* females (white dots) and males (black dots) from Brabant.

Physiology

In total, 97 individual females were tested: 49 females from Brabant and 48 females from Meijendel. All females were weighed and amounts of proteins ($n_{BR} = 49$, $n_{MD} = 48$), lipids ($n_{BR} = 48$, $n_{MD} = 48$), sugars ($n_{BR} = 48$, $n_{MD} = 48$) and glycogen ($n_{BR} = 49$, $n_{MD} = 48$) were measured.

Females from Brabant were significantly heavier than females from Meijendel (Fig. 4.5; mean weight = 799.66 ± 24.50 and 692.30 ± 19.25 µg, respectively; $F_{1,95} = 11.81$, $p = 0.0009$). There was a significant effect of the interaction between population and clutch size on female weight ($F_{1,78} = 9.60$, $p = 0.003$). Within Brabant, clutch size had a significant effect on female weight: females from smaller clutches were heavier than females from larger clutches (Fig. 4.5; $F_{1,32} = 49.92$, $p < 0.0001$). Within Meijendel, there was no significant effect of clutch size on female weight (Fig. 4.5; $F_{1,46} = 0.22$, $p = 0.64$).

Females from Brabant had significantly more proteins than females from Meijendel (Fig. 4.6; mean = 5.85 ± 0.23 and 5.14 ± 0.22 µg, respectively; $F_{1,95} = 4.88$, $p = 0.03$). However, this was due to the significant effect of female weight on the amount of proteins (overall: $F_{3,93} = 51.93$, $p < 0.0001$; weight: $F_{1,95} = 153.36$, $p < 0.0001$). There was no significant difference in the relative amount of proteins between the populations (Fig. 4.6; $F_{1,94} = 0.45$, $p = 0.50$). The interaction between female population and female weight had no significant effect on the amount of proteins ($F_{1,93} = 1.71$, $p = 0.19$). Removing outliers did not change the results.

There was no significant difference in the amount of lipids between females from Brabant and Meijendel (Fig. 4.7; mean = 68.65 ± 4.65 and 62.35 ± 3.84 µg, respectively; $F_{1,94} = 1.09$, $p = 0.30$), but there was a significant effect of female weight on the amount of lipids (overall: $F_{3,92} = 33.50$, $p < 0.0001$; weight: $F_{1,94} = 94.43$, $p < 0.0001$). Also, there was no significant difference in the relative amount of lipids between the populations (Fig. 4.7; $F_{1,93} = 3.94$, $p = 0.05$). The interaction between female population and female weight had no significant effect on the amount of lipids ($F_{1,92} = 0.12$, $p = 0.73$). Removing outliers did not change the results.

There was no significant difference in the amount of sugars between females from Brabant and Meijendel (Fig. 4.8; mean = 5.02 ± 1.46 and 4.43 ± 1.03 µg, respectively; $F_{1,94} = 0.11$, $p = 0.74$) and there was no significant effect of female weight on the amount of sugars (overall: $F_{3,92} = 0.40$, $p = 0.76$; weight: $F_{1,94} = 0.06$, $p = 0.81$). Also, there was no significant difference in the relative amount of sugars between the populations (Fig. 4.8; $F_{1,93} = 0.19$, $p = 0.66$). The interaction between female population and female weight had no significant effect on the amount of sugars ($F_{1,92} = 0.94$, $p = 0.34$). Removing outliers did not change the results.

There was no significant difference in the amount of glycogen between females from Brabant and Meijendel (Fig. 4.9; mean = 27.83 ± 1.61 and 28.27 ± 1.19 µg, respectively; $F_{1,95} = 0.05$, $p = 0.83$), but there was a significant effect of female weight on the amount of glycogen (overall: $F_{3,93} = 11.29$, $p < 0.0001$; weight:

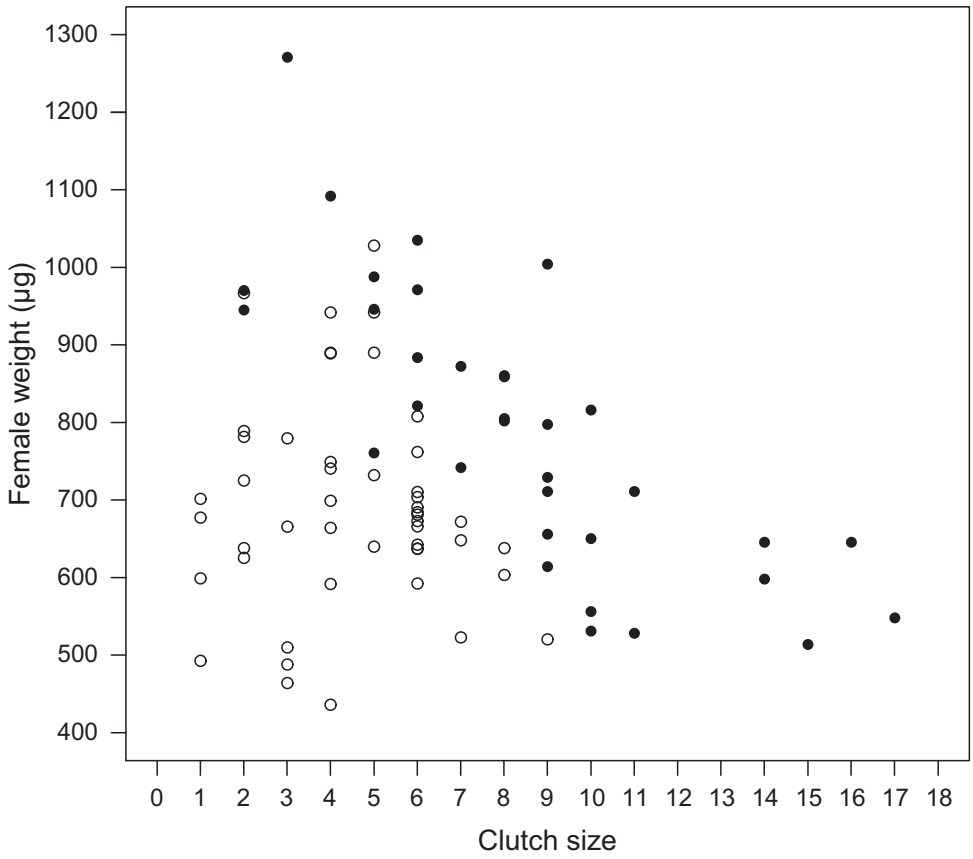

Figure 4.5: Relation between female weight (µg) and clutch size (number of wasps per host pupa) for *Tetrastichus coeruleus* females from Brabant (black dots) and Meijendel (white dots).

$F_{1,95} = 28.29$, $p < 0.0001$). Due to the significant effect of female weight, there was a significant difference in the relative amount of glycogen between the populations (Fig. 4.9; $F_{1,94} = 4.75$, $p = 0.03$); females from Meijendel had relatively more glycogen than females from Brabant. The interaction between female population and female weight had no significant effect on the amount of glycogen ($F_{1,93} = 0.06$, $p = 0.81$). Removing outliers did not change the results.

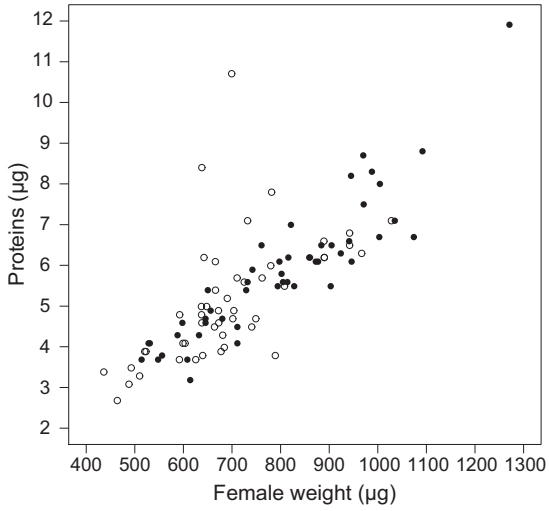

Figure 4.6: Relation between amount of proteins (µg) and female weight (µg), for *Tetrastichus coeruleus* females from Brabant (black dots) and Meijendel (white dots).

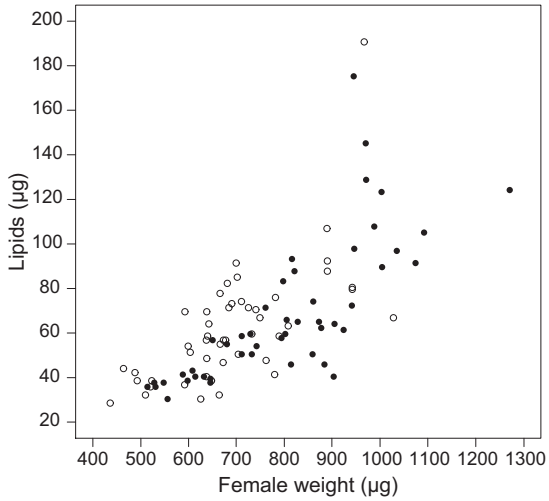

Figure 4.7: Relation between amount of lipids (µg) and female weight (µg), for *Tetrastichus coeruleus* females from Brabant (black dots) and Meijendel (white dots).

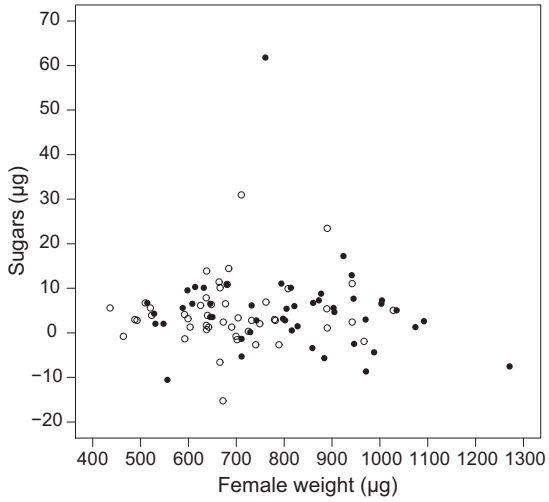

Figure 4.8: Relation between amount of sugars (µg) and female weight (µg), for *Tetrastichus coeruleus* females from Brabant (black dots) and Meijendel (white dots).

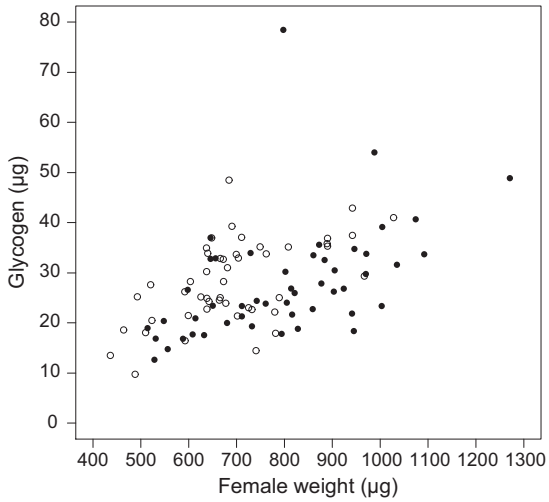

Figure 4.9: Relation between amount of glycogen (µg) and female weight (µg), for *Tetrastichus coeruleus* females from Brabant (black dots) and Meijendel (white dots).

Discussion

We compared life history traits between two populations of *T. coeruleus* that differ in ecology, *Wolbachia* infection and mode of reproduction. Females from Brabant laid larger clutches, had longer life spans and were heavier than females from Meijendel. Furthermore, females from Brabant had lower levels of glycogen per unit body weight than females from Meijendel.

The finding that clutch sizes are larger and female body weight is higher in Brabant than in Meijendel seems paradoxical. Given that individuals from larger clutches have to share the same amount of resources with more siblings, parasitoid wasps emerging from larger clutches are expected to be smaller than those emerging from small clutches. Indeed, we found a negative correlation between clutch size and female weight in Brabant, although not in Meijendel. A possible explanation for the larger clutches and heavier females is that hosts may be larger in Brabant than in Meijendel. Larger hosts will have more resources available and either more or larger parasitoid wasps may emerge from one host pupa. Unfortunately, we have no data on host size in these populations. Alternatively, wasps from Brabant may be more efficient in extracting resources from their host than wasps from Meijendel. Regardless of the cause of the difference in body weight, larger individuals are expected to have a longer life span than smaller ones, because they have more resources available. In agreement with this, females from Brabant were heavier and lived longer than females from Meijendel.

We found a difference in resource allocation only for glycogen. Females from Meijendel contained higher relative glycogen levels than females from Brabant. Levels of other resources (lipids, proteins and sugars) did not differ between females from Brabant and Meijendel after correcting for body weight. Glycogen is used for maintenance, in particular short-term activities (Rivero & Casas 1999). The higher glycogen levels suggest that females from Meijendel invest more in short-term activities, such as flight (Rivero & Casas 1999).

Overall, the two populations appear to employ markedly different life history strategies. Females from Brabant invest more heavily in survival, body size and clutch size, whereas females from Meijendel seem to be more active. The populations from Brabant and Meijendel differ in ecology, *Wolbachia* infection and mode of reproduction. Each of these differences may potentially account for the differences in life history traits we observed. For example, the spatial and temporal availability of hosts differs between Brabant and Meijendel. On agricultural fields in Brabant, asparagus plants occur close together and in high numbers. While hosts may sometimes be abundant, their distribution is rendered unpredictable due to spraying of the asparagus plants with insecticides. This may select for a life history strategy in which females grow large, so they can live longer and exploit available hosts optimally when they are encountered. In natural areas, asparagus plants occur in lower numbers and densities, so females may need to fly more between plants in

search of hosts. However, this is only one of multiple scenarios that may explain the observed differences in life history traits. Furthermore, the direction of causality, if any, cannot be ascertained based on our current data. It is possible that the ecology in Brabant selects for a life history strategy that is less compatible with parthenogenesis than with sexual reproduction, giving uninfected wasps a competitive advantage. Further sampling and experimentation are needed to determine if and how the observed life history differences are related to differences in ecology, mode of reproduction or *Wolbachia* infection.

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