Research

Oikos 119: 533–541, 2010
doi: 10.1111/j.1600-0706.2009.18001.x,

Subject Editors: Tim Benton and Michael Bonsall. Accepted 29 July 2009

Friendly food for fitter flies? – Influence of dietary microbial species on food choice and parasitoid resistance in *Drosophila*

Christiana Anagnostou, Emily A. LeGrand and Marko Rohlfs

C. Anagnostou (canagnostou@zoologie.uni-kiel.de), E. A. LeGrand and M. Rohlfs, Zoological Inst., Dept of Evolutionary Ecology and Genetics, Christian-Albrechts-Univ. of Kiel, Am Botanischen Garten 1-9, D–24118 Kiel, Germany.

Nutrition fuels any activity performed by an organism and has been shown to affect its ability to withstand pathogens and parasites. Furthermore, animals over a wide range of taxa have been shown to exhibit a choice of foods and nutrients that are beneficial to their fitness. Saprophagous animals most often feed on microorganisms growing on dead organic matter rather than the organic matter itself. Various yeast species play an important role in both larval and adult nutrition of saprophagous *Drosophila melanogaster*. We hypothesised the dietary microbial species to affect life-history traits of *D. melanogaster*, including the ability to fend off parasitoids, and larvae to prefer to devour those yeast species beneficial to their development and immunocompetence. Particular yeast species known to be associated with *D. melanogaster* could be shown to have a substantial influence on various larval and adult fitness traits including the ability to encapsulate eggs of the parasitoid wasp *Asobara tabida*. It also turned out that larvae chose to devour those yeast species which supported their ability to encapsulate parasitoid eggs. Which yeast species was preferred and had a beneficial impact on encapsulation ability, was subject to inter-individual variability within the investigated population, hinting at the existence of an adaptive heritable variability regarding individual choice and salubriousness of food. The results suggest that the dietary microbial species of saprophagous insects may influence the resistance against parasitoid attacks and thus the outcome of the interaction between a saprophagous host and its parasitoids.

"One cannot think well, love well, sleep well if one has not dined well" the author Viginia Woolf is supposed to have uttered once. Actually, nutrition fuels any organismal activity and everything in nature seems to centre on food and how to get it (Hernes 2000). Since pathogens and parasites are omnipresent and may pose drastic fitness costs on their hosts (Wilson 2005), it may be crucial for an organism to invest (nutritional) resources into defence mechanisms. It has long been recognised that there is a close relationship between the host's nutritious state and its ability to overcome infection (Chandra 1996). Besides the more obvious detrimental consequences on immune responsiveness resulting from starvation or lack of food (Chandra 1996, 1997, 1999, 2002, Brown et al. 2000, Siva-Jothy and Thompson 2002, Houston et al. 2007, Campero et al. 2008), the quantity as well as the quality of specific nutritional resources such as carbohydrates, proteins, lipids or micronutrients, may influence resistance to pathogen attack (Rivera et al. 2003, Li et al. 2007, Lee et al. 2008). The host's nutritional status may play a pivotal role in host–parasite relationships, influencing the host's defence mechanisms and resource allocation to immune defences (Siva-Jothy and Thompson 2002, Lee et al. 2006), but also directly influencing the parasite, that usually utilises the host's tissues and body fluids as food source, which themselves are a product of the food consumed by the host (Krasnov et al. 2005, Seppälä et al. 2008). Thus, nutrition may modulate both the parasite's virulence and the host's resistance (Coop and Kyriazakis 2001, Logan et al. 2005).

Animals leading a saprophagous lifestyle play an essential role as decomposers of organic matter. Their diet usually consists of not only the often nitrogen-poor dead organic matter itself, but also the associated microorganisms (Begon et al. 2005). It remains to be investigated, whether different microbial species in the diet of saprophagous animals may influence their ability to fend off parasite attack. To test this, we looked at larvae of saprophagous *Drosophila melanogaster* that develop in injured or decaying plant material usually rich in microorganisms such as bacteria and fungi, especially yeasts. It is mainly these microorganisms that act as actual nutrient resource, processing the plant raw material into important dietary factors (Starmer 1981). Yeasts are considered as a major food source for the majority of species of *Drosophila* in both adult and larval stages (Begon 1982). The presence of yeast itself, irregardless of a particular species, is crucial for the fly's successful development and it also turned out that larvae parasitised by the cynipid wasp *Leptopilina boulardi* and deprived of yeast immediately after parasitisation, encapsulated a significantly lower percentage of the parasitoid's eggs than hosts transferred to a medium with yeast (Vass and Nappi 1998). Here, we hypothesised, (1) that different yeast species may influence life-history traits

(such as survival, development time and body weight) of unparasitised larvae, and (2) that not only the presence of yeast itself but also different yeast species may influence the ability of *D. melanogaster* to encapsulate eggs of the braconid wasp *Asobara tabida*. As model yeasts we chose yeast species reported to have been isolated from *Drosophila* spp. and various breeding sites (Begon 1982, Kearny 1982).

According to the findings, that encapsulation ability in *D. melanogaster* is at least partially related to the number of circulating haemocytes in the haemolymph (Eslin and Prévost 1995), we expected (3) to find an increased number of haemocytes in the haemolymph of those *D. melanogaster* larvae that had fed from the yeast species enhancing their ability to encapsulate parasitoid eggs. To test this we chose two yeast species shown to have a differing influence on the encapsulation ability of *D. melanogaster* larvae parasitised by *A. tabida* and counted the number of all types of haemocytes (total haemocyte count, THC) circulating in the haemolymph. We included unparasitised larvae into the experiment to be able to discriminate, whether consuming a specific yeast species would generally enhance THC, not only after an immunological challenge.

Which food is consumed by the host is often subject to the host's choice of food. Animals over all taxa have been shown to continuously regulate their food intake, whereby their choice of foods or nutrients are non random and beneficiary to their fitness (Waldbauer and Friedman 1991). Infection with parasites and pathogens may have a drastic impact on the host's metabolism and behaviour, including a change in feeding behaviour, due to manipulation of host behaviour by the parasite, modified nutritional requirements, activation of defence mechanisms or energetic constraints imposed on an infected organism (Thompson and Kavaliers 1994, Barber et al. 2000, Jog and Watve 2005). In a number of studies, parasitised animals exhibited compensatory shifts in diet choice and consumption, which also influenced the developmental success of the parasite (Singer et al. 2004, 2009, Thompson and Redak 2005, 2008, Thompson et al. 2005, Lee et al. 2006). Already in some early studies it was observed, that different species of unparasitised *Drosophila* showed preferences for different species of yeasts and that the yeasts preferred by the larvae tended to be those best supporting their larval growth (Lindsay 1958, Cooper 1960, Fogleman et al. 1981, Dorsch 2007). In our study we hypothesised (4) that unparasitised larvae of *D. melanogaster* show a preference for yeast species beneficial to their larval development, (5) that parasitised larvae show a preference for yeast species beneficial to their ability to encapsulate eggs of *A. tabida*, and (6) that the preference of unparasitised and parasitised larvae may not be identical.

That individuals often differ consistently in their behaviour has been shown in several animal taxa including humans, with differences appearing to have a heritable basis (Gosling 2001, Bell 2007). Inter-individual differences within animal populations are also argued to explain some of the large observed variation in rates of energy metabolism in animals (Careau et al. 2008). Considering this, we wanted to test the hypothesis, (7) that *D. melanogaster* larvae also exhibit inter-individual differences regarding their choice of particular yeast species and the influence on immune defence posed by these yeasts. To investigate this we chose two yeast species shown to have a differing but not always consistent influence on the encapsulation ability of *D. melanogaster* larvae parasitised by *A. tabida*. Larvae were allowed to choose the yeast of their liking. For further larval development they were then transferred either on the yeast species chosen or the one refused (in the following this test is referred to as 'crosswise transfer').

Material and methods

Fly and parasitoid cultures

The *Drosophila melanogaster* population originated from 20 isofemale lines caught in Kiel, northern Germany, in 2003. The population had undergone approx. 70 generations before it was used in the experiments. The population of *Asobara tabida* stemmed from animals caught in the surrounding area of Leiden Univ. and provided by Jaques van Alphen from University Leiden, NL, in 1998, and was cultured on *D. melanogaster*.

Yeast species and cultivation

The yeast species chosen were *Cryptococcus albidus* (DSM 70215), *Kluyveromyces lactis* (DSM 4909), *Metschnikowia pulcherrima* (DSM70321), *Pichia toletana* (DSM 70390) and *Saccharomyces cerevisiae* (DSM 70449), obtained from the DSMZ (German Resource Centre for Biological Material) in Braunschweig. We cultivated the yeasts in malt extract broth out of glycerine stocks, incubated them over night at 30°C on a shaker, plated them on malt extract agar and incubated them at 27°C for three days. We then washed off the colonies with sterile Ringer's solution and pipetted the suspension into 50 ml conic flasks. Suspensions were centrifuged (3000 rpm, 10 min) and the supernatant was decanted. To gain the final experimental suspension of 50%, we resuspended the pellet in the adequate amount of Ringer's solution.

Media

Axenic medium for cultivating *Drosophila* contained 62.5 g each of sugar, corn flour and yeast extract, 12.5 g agar and one litre tap water. After autoclaving and cooling down, 30 ml nipagin, 10 ml sorbic acid, 10 ml chloramphenicol (each with a concentration of 10% in 100% ethanol) and 2 ml nystatin-dihydrate (5% in dimethylsulfoxide) were added. Flies were kept in a climate chamber at 25°C and a 16:8 h L:D cycle. *A. tabida* was cultured at the same L:D cycle but merely at 20°C.

For the experiments, we decided to choose a substrate more similar to those *D. melanogaster* may encounter in the wild, and picked bananas. Depending on the particular requirements of each experiment, we prepared four different banana-based media (e.g. with higher amounts of agar, which hindered larvae from burrowing too deeply into the substrate and made it easier for us to find them again): Banana medium I: finely crushed banana mixed with the same amount of tap water and 12.5 g agar per litre banana-water mixture plus 30 ml nipagin and 10 ml chloramphenicol per litre autoclaved medium. Banana medium II: same as Banana

medium I without antibiotics. Banana medium III: same as Banana medium I but with 25 g agar per litre banana-water mixture. Banana medium IV: same as banana medium III without antibiotics**.** Nipagin agar: 20 g agar mixed with one litre tap water plus 30 ml nipagin per litre autoclaved medium.

Preparation of larvae

We allowed the flies to oviposit for approx. 12 h into culturing flasks, dechorionated the eggs with 50% sodium hypochlorite and transferred them with a sterile brush onto a petri dish containing Banana medium I. For respiration the lid was provided with a small hole covered with nylon gaze and the eggs were incubated in a climate chamber at 25°C and a 16:8 h L:D cycle. The freshly hatched 1st instar larvae were washed onto a small nylon gaze sieve (mesh size 50 µm) and could then be transferred with a fine brush.

Gaining of parasitised larvae

One method used was to permanently observe *A. tabida* during oviposition ('observational method'): We transferred several freshly hatched 1st instar larvae into a 35 mm petri dish filled with Banana medium III, added a female parasitoid and observed it during oviposition. Larvae that were punctured by the ovipositor of *A. tabida* but were most likely rejected (i.e. no egg was laid), were not included in the experiment. Similar to van Alphen and Janssen (1982) as well as Wertheim et al. (2005), larvae were defined as 'rejected' when the ovipositor was inserted for less than 10 s. Unrejected larvae were removed immediately after oviposition and transferred onto a fresh petri dish. We allowed each parasitoid to parasitise approx. 10 larvae and exchanged it afterwards. To avoid possible effects of single parasitoids, we evenly distributed larvae parasitised by one parasitoid over all treatments.

The other method we used is based on the one described by Fellowes et al. (1998) and Kraaijeveld et al. (2001, 2008), and does not require to permanently observe the parasitoid during oviposition and is thus less time-consuming ('non-observational method'): we transferred 20 larvae each onto a petri dish filled with Banana medium III and allowed two female *A. tabida* to parasitise the larvae for approx. one and a half hours.

Experimental proceeding

Basic experimental design for testing hypotheses (1)–(3) and (7): 50 µl each of the 50% yeast suspension were pipetted into the prepared test tubes filled with Banana medium II. For gaining parasitised larvae we used the observational method. We transferred one parasitised and one unparasitised larva, respectively, each into the tube with a fine brush, covered the tubes with dental rolls and incubated them in a climate chamber at 25°C and a 16:8 h L:D cycle. The number of replicates prepared was 40 for unparasitised larvae and 100 for parasitised larvae (to leave enough replicates in case of a low parasitisation rate). In parasitised larvae, survival until pupation and encapsulation ability were measured. The development of unparasitised larvae was followed until eclosion and adult flies were dried and weighed.

Experimental design for testing hypothesis (3) (haemocyte count): We followed the basic design as described above with the addition of washing larvae out of the test tube for haemocyte count, after the time spans of 6, 12 and 24 h, respectively, had elapsed. The number of replicates prepared was 40 for unparasitised larvae and 80 for parasitised larvae (to leave enough replicates in case of a low parasitisation rate). The only exception was at the time span of 6 h after parasitisation, where we also prepared 40 replicates for parasitised larvae.

Experimental design for testing hypotheses (4)–(6) (food choice of unparasitised and parasitised larvae): The yeast species *K. lactis*, *M. pulcherrima* and *S. cerevisiae* were offered (two at a time) in the two yeast combinations *K. lactis* and *M. pulcherrima* as well as *K. lactis* and *S. cerevisiae*. As food choice arenas we filled 55 mm petri dishes with 8 ml nipagin agar, bored two holes (diameter 8 mm) in a distance of 30 mm from each other (calculated from the inner margin of each hole) and filled them with Banana medium IV. Twenty-four hours prior to each food choice experiment we pipetted 1 µl of a 50% yeast suspension onto each of the banana patches and incubated the arenas over night in a climate chamber at 25°C and a 16:8 h L:D cycle. For the food choice experiment, we arranged the arenas in the way that each yeast species in a combination of two was alternately positioned on the left and on the right side in order to avoid effects of position or light conditions on larval food choice. We prepared 10 replicates for each yeast combination as well as unparasitised and parasitised larvae. We then transferred 20 freshly hatched larvae per replicate into the food choice arenas and placed them near the edge of the petri dish roughly equidistant to each banana-yeast patch. Larvae were then left for one hour. Immediately after the hour had elapsed, we put the arenas under a stereo microscope and noted the number of larvae on each of the banana-yeast patches. Parasitised larvae had to be put into the freezer (–20°C) to 'freeze' their position on the patches for later counting and dissection in order to verify parasitisation (presence of parasitoid egg or capsule). We only included actually parasitised larvae in the statistical analysis. An actual effect on larval physiology and behaviour may occur not until several hours after parasitisation (encapsulation usually takes place between 3 and 72 h after parasitisation, Wertheim et al. 2005) and may also vary considerably among individual larvae. Food choice, however, should take place within a time span where it still makes sense for larvae to choose food more advantageous to their parasitised state. Based on preliminary food choice tests carried out after 3, 6 and 24 h after parasitisation (Anagnostou 2008), we decided to choose the time span of 24 h, where the larval response was most pronounced. For gaining parasitised larvae we used the non-observational method. During the time span after parasitisation before the food choice experiments were carried out, parasitised as well as unparasitised larvae were incubated on petri dishes (diameter 55 mm) containing Banana medium III. The food choice experiment with the yeast combination *K. lactis* and *S. cerevisiae* was repeated with 51 replicates for unparasitised as well as parasitised larvae.

For the editing of data, we calculated a delta value. Imagine as an example the two banana patches A and B with the two yeast species A and B. The number of larvae found on

patch B was subtracted from the number of larvae found on patch A and was then divided by the number of all larvae that had made a food choice (i.e. the number of larvae found on patch A plus the number of larvae found on patch B). Delta values between –1 and +1 may result. If delta is 0, there was no preference for any of the two yeasts (i.e. the number of larvae on patch A was identical to that on patch B). If delta is +1, yeast A was strongly preferred (all larvae that had made a choice were found on patch A). If delta is –1, yeast B was strongly preferred. Values between 0 and +1 and between –1 and 0, respectively, hint at an increasing preference for yeast A or yeast B.

Experimental design for testing hypothesis (7) (crosswise transfer): We followed the basic design as described above and used the non-observational method for gaining parasitised larvae. The yeast species combination *K. lactis* and *S. cerevisiae* was chosen. We transferred one larva each into the arena and observed the larvae until they had made a choice. Just before they were about to enter the yeast patch, we seized them with a brush and transferred them into a prepared experimental tube with Banana medium II and a 50% suspension of the yeast of their choice. The next larva that had made the same choice was transferred into a tube containing the yeast species it had refused. The same was done for larvae that had chosen to devour the other of the two offered yeast species. Eighty replicates per yeast species chosen and yeast species developed on, were prepared.

Determination of encapsulation ability

Approximately two days after pupation of parasitised larvae, we collected the pupae from the test tubes, dissected them under a stereo microscope and noted the absence or presence of developing flies, parasitoids and capsules. We included only those individuals in the analysis that either contained a live unencapsulated parasitoid larva (parasitised, unsuccessful defence) or a capsule (parasitised, successful defence). Very often parasitised flies contained a live parasitoid larva and rests of a former capsule, which the parasitoid had succeeded in breaking out of. These flies were assigned as being parasitised and not exhibiting successful defence. Unparasitised flies were excluded from the analysis.

Haemocyte count

The method we used for obtaining circulating haemocytes was a combination of the methods carried out by Lanot et al. (2001) and Huang et al. (2005). After the time spans of 6, 12 and 24 h after parasitisation had elapsed, we washed each larva out of the test tube onto a nylon gaze sieve, washed it thoroughly in PBS buffer and put it on filter paper to dry. It was then bled on a microscopic slide. We dissected the carcass of parasitised larvae to look for a parasitoid egg (or capsule) in order to verify parasitisation. Haemolymph specimens were allowed to dry and were fixed in 100% methanol for 10 min. After drying, specimens were dyed with 10% Giemsa stain for one h. We counted all types of haemocytes at a magnification of 480. The whole specimen was scanned by meandering through it. This was repeated four times and the arithmetic mean finally used for further calculation. The above method only renders underestimated haemocyte numbers, but since we treated specimens in the same way and compared them to each other, no absolute numbers were required.

Statistical analyses

All statistical analyses were carried out with R 2.5.0. We tested proportional data (survival and encapsulation ability) and data on development time, dry weight, haemocyte count as well as the delta value (response variables) using generalised linear models (for proportional data: family: binomial; link: logit; for continuous data: family: poisson or quasipoisson; link: identity), with yeast species as linear predictor. Regarding the food choice experiment, we used single-sample t-tests (for normally distributed and variance-homogenous data) as well as GLMs (family: quasipoisson; link: identity) to test for a significant difference in the number of larvae found on each of the two yeast patches.

Results

Developmental success and encapsulation

The dietary yeast species could be shown to influence certain life history traits of unparasitised as well as parasitised *D. melanogaster* larvae and emerging adults. The species *K. lactis* and *S. cerevisiae* appeared to have the most beneficial influence on various fitness traits, followed by the species *C. albidus*, *P. toletana* and *M. pulcherrima*.

Results for unparasitised larvae

Microbial diet had no significant effect on the proportion of unparasitised larvae surviving until eclosion ($F_{4,195} = 2.2504$, $p = 0.0651$) (data not shown). Development time was significantly affected ($F_{4,186} = 14.753$, $p < 0.001$) (cf. Fig. A1 A in the Supplementary material Appendix 1) as well as female dry weight of adult flies ($F_{4,91} = 12.764$, $p < 0.001$), but there was no significant effect on male dry weight ($F_{4,90} = 1.8204$, $p = 0.1318$) (cf. Fig. A1 B in the Supplementary material Appendix 1).

Results for parasitised larvae

Microbial diet had no significant effect on the proportion of parasitised larvae surviving until pupation ($F_{4,419} = 1.7334$, $p = 0.1416$) (data not shown). Encapsulation ability, however, was significantly influenced by the different dietary yeast species ($F_{4,358} = 3.2049$, $p = 0.01$). Some yeasts had a more beneficial influence on encapsulation ability (e.g. *K. lactis* or *M. pulcherrima*) than others (e.g. *S. cerevisiae*, *C. albidus* or *P. toletana*) (Fig. 1).

Haemocyte count

At none of the three time spans after parasitisation that the total haemocyte count was carried out, a significant difference in the number of circulating haemocytes could be found between larvae having fed on *K. lactis* and those having fed on *S. cerevisiae*, neither in unparasitised (six h after paras.: $F_{1,55} = 0.1161$, $p = 0.7346$; 12 h after paras.: $F_{1,55} = 0.2059$, $p = 0.6518$; 24 h after paras.: $F_{1,56} = 0.0001$, $p = 0.9928$) nor parasitised larvae (6 h after paras.: $F_{1,50} = 0.022$, $p = 0.8827$;

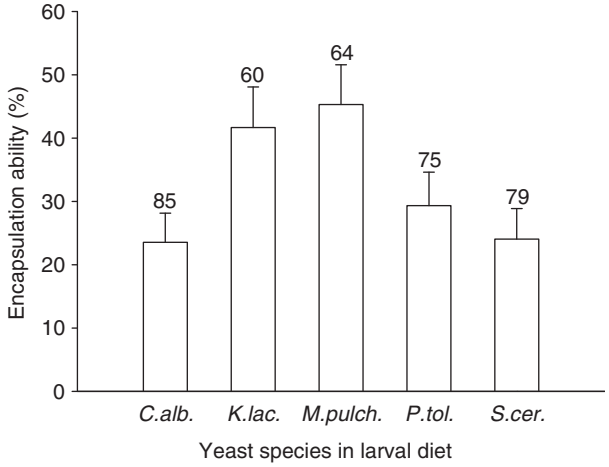

Figure 1. Mean (+ SE) encapsulation ability of *Drosophila melanogaster* larvae against the parasitoid wasp *Asobara tabida* depending on the yeast species in the larval diet. Numbers above columns = number of replicates. Abbreviations of yeasts: *Cryptococcus albidus* (*C.alb.*), *Kluyveromyces lactis* (*K.lac.*), *Metschnikowia pulcherrima* (*M.pulch.*), *Pichia toletana* (*P.tol.*), *Saccharomyces cerevisiae* (*S.cer.*).

12 h after paras.: $F_{1,124} = 0.0718$, p = 0.7891; 24 h after paras.: $F_{1,130} = 0.9674$, p = 0.3272) (Fig. 2).

Diet choice

Yeast combination K. lactis and M. pulcherrima

Unparasitised larvae exhibited a significant preference for *K. lactis* over *M. pulcherrima* (t = 7.0316, DF = 9, p < 0.0001). Also parasitised larvae significantly preferred *K. lactis* over *M. pulcherrima* (t = 7.8117, DF = 9, p < 0.0001), even more so than unparasitised ones ($F_{1,18} = 4.552$, p = 0.0469) (Fig. 3A).

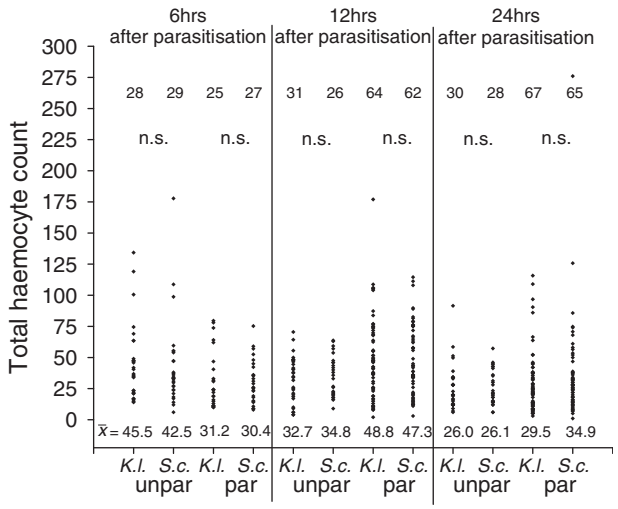

Figure 2. Total haemocyte count of unparasitised (unpar) as well as parasitised (par) larvae fed on *Kluyveromyces lactis* and *Saccharomyces cerevisiae* at 6, 12 and 24 h after parasitisation. Abbreviations of yeasts: *Kluyveromyces lactis* (*K.l.*), *Saccharomyces cerevisiae* (*S.c.*). Numbers above scatter plots = number of data points (replicates). Numbers below scatter plots = mean values. Significance level: n.s. = not significant.

Yeast combination K. lactis and S. cerevisiae

In unparasitised larvae, no preference for any of the two yeast species could be observed (t = –0.3389, DF = 9, p = 0.7424).

Parasitised larvae significantly preferred *K. lactis* over *S. cerevisiae* (t = 3.2313, DF = 9, p = 0.0103); the difference between unparasitised and parasitised larvae was, however, not significant ($F_{1,18} = 4.1289$, p = 0.05718) (Fig. 3B). When the food choice experiment with the yeast combination *K. lactis* and *S. cerevisiae* was repeated, unparasitised larvae as well as parasitised larvae preferred *K. lactis* over *S. cerevisiae* (unparas. larvae: t = 14.7073, DF = 101, p < 0.0001; paras. larvae: t = 39.35, DF = 101, p < 0.0001), and the difference between unparasitised and parasitised larvae was significant ($F_{1,202} = 4.503$, p = 0.03505) (Fig. 4).

Crosswise transfer

When parasitised larvae were given the chance to feed from the yeast they had chosen, encapsulation ability was significantly enhanced compared to when they were forced to feed from the yeast they had refused (Fig. 5). This was true both for larvae that had chosen *K. lactis* ($F_{1,52} = 4.5025$, p = 0.03863) and those that had chosen *S. cerevisiae* ($F_{1,27} = 7.047$, p = 0.01315).

Discussion

Since the 'father of immunology' Ilya Metchnikov, investigating the microbiota and the immune system have increased significantly in recent years (Huffnagle and Noverr 2008). Even though a vast number of microbiota may be initiators

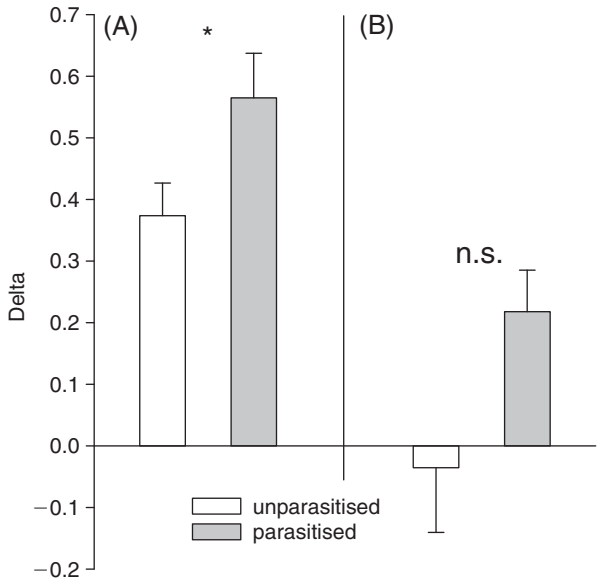

Figure 3. Mean (+ SE) delta values of food choice experiment with unparasitised and parasitised larvae 24 h after parasitisation, for the yeast combinations *Kluyveromyces lactis* and *Metschnikowia pulcherrima* (A) as well as *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (B). Delta values > 0 hint at a preference for *K.lac.*, delta values < 0 hint at a preference for either *M.pulch.* (A) or *S.cer.* (B). No. of replicates = 10. Significance levels: n.s. = not significant, *p < 0.05.

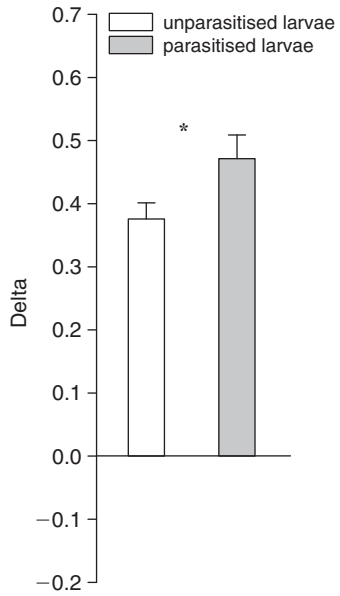

Figure 4. Mean (+ SE) delta values of repeated food choice experiment with unparasitised and parasitised larvae 24 h after parasitisation, for the yeast combination *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. Delta values > 0 hint at a preference for *K.lac.*, delta values < 0 hint at a preference for *S.cer.* No. of replicates = 51. Significance level: *p < 0.05.

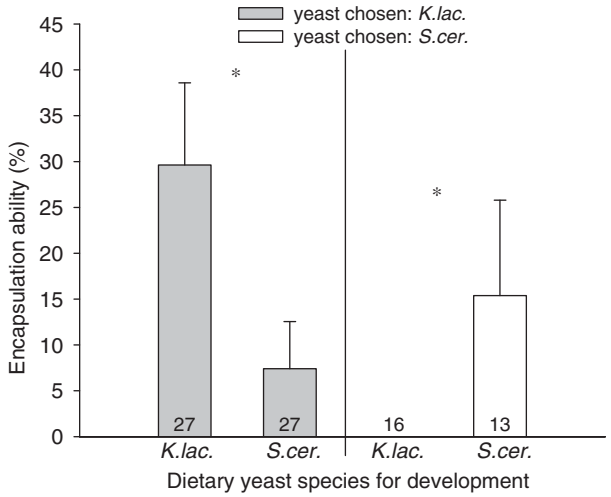

Figure 5. Mean (+ SE) encapsulation ability of *Drosophila melanogaster* larvae against the parasitoid wasp *Asobara tabida* depending on the yeast species chosen and the one consumed during subsequent development. No. within columns = number of replicates. Abbreviatons of yeasts: *Kluyveromyces lactis* (*K.lac.*), *Saccharomyces cerevisiae* (*S.cer.*). Significance level: *p < 0.

of diseases, they may also pose a major positive regulatory force for immune responses (Noverr and Huffnagle 2004). As a saprophagous insect that fulfils its development on decaying organic matter, *D. melanogaster* encounters a plethora of different microorganisms. Especially yeast species pose the most important food source for larval as well as adult *D. melanogaster* (Begon 1982). The findings of our first experiment appear to support our hypotheses (1) and (2): In addition to confirming the observation in former studies, that different species of yeasts may have a different influence on the developmental success of unparasitised *Drosophila* larvae (Lindsay 1958, Cooper 1960, Dorsch 2007), we could show that also parasitised *D. melanogaster* larvae may experience an influence of dietary yeast species on their ability to encapsulate eggs of the parasitoid wasp *A. tabida*. So far, the influence of nutritional deprivation as well as host plant species or sugar concentration on encapsulation, melanisation and nodule formation has been investigated (Feder et al. 1997, Suwanchaichinda and Paskewitz 1998, Koella and Sorensen 2002, Siva-Jothy and Thompson 2002, Ojala et al. 2005). That particular dietary microorganisms may influence the encapsulation ability of a saprophagous insect adds a novel aspect.

As they move in and on their food, *D. melanogaster* larvae, similar to many other dipteran especially cyclorrhaphous larvae, perceive a number of food-associated stimuli, using a mixture of gustation and olfaction (Cobb 1999). Lindsay (1958), as well as Cooper (1960) and Dorsch (2007) had already observed that unparasitised larvae of different species of *Drosophila* showed decided preferences when given the choice of several yeast species, and that they tended to prefer those yeast species which best supported their larval growth. In this study it could additionally be observed, that *D. melanogaster* larvae parasitised by *A. tabida* may exhibit a preference for yeast species that had turned out to beneficially influence their encapsulation ability, and this preference may differ from that exhibited by unparasitised larvae. These findings support our hypotheses (4), (5) and (6). Thompson et al. (2005) and Singer et al. (2009) found that feeding and nutrient intake may differ markedly between unparasitised and parasitised larvae and depend on the ratio of digestible protein and carbohydrate and on secondary metabolites. The different food preferences observed in parasitised *D. melanogaster* larvae may have been due to nutritional requirements altered by parasitisation. The requirements may, e.g. differ especially in respect to micronutrients, and may be met in a better way by particular yeast species (e.g. *K. lactis*). According to Fekete and Kellems (2007), there is increasing evidence that the concentration of trace elements required for healthy animals are often below what is required for animals experiencing an immunological challenge. The presence of sterols may also have influenced diet choice: Yeast species may vary greatly in their sterol content, e.g. ergosterol (Bills et al. 1930). Sterols are essential compounds in an insect's diet, yet insects are not able to synthesise them de novo (Behmer and Nes 2003, Nagata et al. 2006).

Regarding the yeast combination *K. lactis* and *S. cerevisiae*, parasitised *D. melanogaster* larvae exhibited a significant preference for *K. lactis*, which had been shown to influence encapsulation ability in a more positive way than *S. cerevisiae*. The difference in preference between unparasitised and parasitised larvae was, however, not significant. When the experiment was repeated with a higher number of replicates, a significant difference did arise; this time, however, also unparasitised larvae exhibited a significant preference for *K. lactis*. The observed variability in yeast preference or the degree of the preference for a specific yeast among both unparasitised and parasitised larvae, raised the question, whether there is an innate behavioural variability within the population that is adaptive. This

hypothesis (7) was supported by the findings, that larvae exhibited higher encapsulation ability when given the chance to develop on the yeast of their liking. Even though there was a strong tendency of *K. lactis*, compared to *S. cerevisiae*, to enhance encapsulation ability in a large proportion of *D. melanogaster* larvae, this may not hold true for all larvae. For a (smaller) proportion of larvae, *S. cerevisiae* may even be the more beneficial yeast species. A naturally occurring intraspecific variation in olfactory response has been found in *D. melanogaster* (Cobb 1999). The individual responses to olfactory stimuli and the individual preferences of larvae may originate from genetically determined individual differences in tolerance and salubriousness of specific food ingredients posed by different species of yeast.

The underlying mechanism of the differing influence of dietary yeast species on encapsulation ability was tried to be revealed by determining the number of circulating haemocytes, since enhanced encapsulation ability is supposed to be positively correlated to haemocyte number (Eslin and Prévost 1998, Kraaijeveld et al. 2001). When *K. lactis* and *S. cerevisiae* were chosen as representative species, no differences in haemocyte number could be found which may have corresponded to the more positive influence of *K. lactis* on encapsulation ability compared to that of *S. cerevisiae*. Thus, we cannot reject the H_0-hypothesis, that yeast diet has no effect on haemocyte number. It may be very likely that a difference in haemocyte number could not be detected due to inter-individual differences within the investigated fly population, with *K. lactis* enhancing Total haemocyte count in one proportion of larvae, *S. cerevisiae* in another. A potential increase in the number of circulating haemocytes may, however, not be the only mechanistic explanation for the differing influence of the yeast species on encapsulation ability. Other features of the immune system, such as the phenoloxidase activity, may just as well have experienced a modulation by the dietary yeast species, as could e.g. be shown in *Artemia* larvae (Rojas-García et al. 2008). Some microbial species in the larval diet may also have stimulated components of the immune system in a way to make it more alert and ready for an actual challenge by a serious pathogen or parasite (Freitak et al. 2007). So called probiotic organisms are reported to possess the ability of modulating the host's immune response, but also of interfering with the ability of another, potentially pathogenic microorganism, to colonise the host (Reid et al. 2001, Macey and Coyne 2005). *Kluyveromyces lactis* could be shown to possess potentially probiotic qualities, due to its ability to adhere to human enterocyte-like Caco-2 cells, whereby adhesion to the intestinal mucosa is considered important for exclusion of pathogens and undesirable bacteria (Kumura et al. 2004).

It has been shown that the physiology of *D. melanogaster* is extremely responsive to diet, and transcriptional response to changes in nutrients may occur over a short time scale (Gershman et al. 2006). Regarding this, it would be interesting to investigate the response in gene expression of unparasitised as well as parasitised larvae after having fed on particular species of yeast, both the one preferred and the one refused, respectively.

Even though a mechanistic explanation to the phenomena observed in this study is still to be found and despite the variability in the influence of particular yeast species on the behaviour and life history traits of *D. melanogaster*, the results suggest, that the microbial species composition of the food of a saprophagous host may influence the outcome of the interaction between the host and its parasitoids. Regarding the observed (heritable) variation around the behavioural or metabolic 'golden mean', with some phenotypes being more fit than others in particular (food) conditions, the dietary microbial species composition may sustain genetic variability within a host population and influence potential coevolutionary processes between host and parasitoid.

Acknowledgements – Many thanks to Leiden Univ. and especially Jaques van Alphen for providing us with individuals of *Asobara tabida* in order to set up our own culture. We thank Christina Griebner for her help with culturing *A. tabida* and preparing basic *Drosophila* culturing media. Many thanks to Jan von Rönn, for his statistical advice and help with R. MR is supported by the DFG research grant (RO 3523/2-1). EG was funded by the DAAD within the scope of the RISE program 2008; she is a Biology student at Carlton College, Minnesota, USA. CA is supported by the state Schleswig-Holstein.

References

Anagnostou, C. 2008. Influence of the microbial environment on the interaction of a saprophagous insect and its parasitoids. PhD thesis. – Christian-Albrechts-Univ. of Kiel, Kiel. <http://eldiss.uni-kiel.de/macau/receive/dissertation_diss_00003491>.

Barber, I. et al. 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. – Rev. Fish. Biol. Fish. 10: 131–165.

Begon, M. 1982. Yeasts and *Drosophila*. – In: Ashburner, M. et al. (eds), The genetics and biology of *Drosophila.* Academic Press, pp. 345–384.

Begon, M. et al. 2005. Ecology: from individuals to ecosystems (4th ed.). – Wiley.

Behmer, S. T. and Nes, W. D. 2003. Insect sterol nutrition and physiology: a global overview. – Adv. Insect Physiol. 31: 1–72.

Bell, A. L. 2007. Future directions in behavioural syndromes research. – Proc. R. Soc. Lond. B 274: 755–761.

Bills, C. E. et al. 1930. Factors determining the ergosterol content of yeast. – J. Biol. Chem. 87: 259–264.

Brown, M. J. F. et al. 2000. Condition-dependent expression of virulence in a trypanosome infecting bumblebees. – Oikos 91: 421–427.

Campero, M. et al. 2008. Correcting the short-term effect of food deprivation in a damselfly: mechanisms and costs. – J. Anim. Ecol. 77: 66–73.

Careau, V. et al. 2008. Energy metabolism and animal personality. – Oikos 117: 641–653.

Chandra, R. K. 1996. Nutrition, immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. – Proc. Natl Acad. Sci. USA 93: 14304–14307.

Chandra, R. K. 1997. Nutrition and the immune system: an introduction. – Am. J. Clin. Nutr. 66: 460–463.

Chandra, R. K. 1999. Nutrition and immunology: from the clinic to cellular biology and back again. – Proc. Nutr. Soc. 58: 681–683.

Chandra, R. K. 2002. Nutrition and the immune system from birth to old age. – Eur. J. Clin. Nutr. 56 (Suppl. 3): 73–76.

Cobb, M. 1999. What and how do maggots smell? – Biol. Rev. 74: 425–459.

Coop, R. L. and Kyriazakis, I. 2001. Influence of host nutrition on the development and consequences of nematode-parasitism in ruminants. – Trends Parasitol. 17: 325–330.
Cooper, D. M. 1960. Food preferences of larval and adult Drosophila. – Evolution 14: 41–55
Dorsch, M. 2007. Einfluss der mikrobiellen Nahrung auf die Entwicklung von *Drosophila*. Diplomarbeit. – Christian-Albrechts-Univ. Kiel.
Eslin, P. and Prévost, G. 1998. Hemocyte load and immune resistance to *Asobara tabida* are correlated in species of the *Drosophila melanogaster* subgroup. – J. Insect Physiol. 44: 807–816.
Feder, D. et al. 1997. Immune responses in *Rhodnius prolixus*: influence of nutrition and ecdysone. – J. Insect Physiol. 43: 513–519.
Fekete, S. Gy. and Kellems, R. O. 2007. Interrelationship of feeding with immunity and parasitic infection: a review. – Vet. Med. 52: 131–143.
Fellowes, M. D. E. et al. 1998. Tradeoff associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. – Proc. R. Soc. Lond. B 265: 1553–1558.
Fogleman, J. C. et al. 1981. Larval selectivity for yeast species by *Drosophila mojavensis* in natural substrates. – Proc. Natl Acad. Sci. USA 78: 4435–4439.
Freitak, D. et al. 2007. Immune system responses and fitness costs associated with consumption of bacteria in larvae of *Trichoplusia ni*. – BMC Biol. 5: 1–13.
Gershman, B. et al. 2006. High resolution dynamics of the transcriptional response to nutrition in *Drosophila*: a key role for dFOXO. – Physiol. Genomics 29: 24–34.
Gosling, S. D. 2001. From mice to men: what can we learn about personality from animal research? – Psychol. Bull. 127: 45–86.
Hernes, G. 2000. Eating to your heart's delight. – Sci. Total Environ. 249: 395–410.
Houston, A. I. et al. 2007. The effect of energy reserves and food availability on optimal immune defence. – Proc. R. Soc. Lond. B 274: 2835–2842.
Huang, L. et al. 2005. The lesswright mutation activates Rel-related proteins, leading to overproduction of larval hemocytes in *Drosophila melanogaster*. – Dev. Biol. 280: 407–420.
Huffnagle, G. B. and Noverr, M. C. 2008. GI microbiota and regulation of the immune system. – Ser. Adv. Exp. Med. Biol. 635, pp. 149.
Jog, M. and Watve, M. 2005. Role of parasites and commensals in shaping host behaviour. – Curr. Sci. 89: 1184–1191.
Kearny, J. N. 1982. Oviposition and feeding on naturally occurring yeasts by *Drosophila* spp., using natural substrates. – Oikos 39: 103–112.
Koella, J. C. and Sorensen, F. L. 2002. Effect of adult nutrition on the melanization response of the malaria vector *Anopheles stephensi*. – Med. Vet. Entomol. 16: 316–320.
Kraaijeveld, A. R. et al. 2001. Basis of the trade off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. – Proc. R. Soc. Lond. B 268: 259–261.
Kraaijeveld, A. R. et al. 2008. Stage-specific sex-differences in *Drosophila* immunity to parasites and pathogens. – Evol. Ecol. 22: 217–228.
Krasnov, B. R. et al. 2005. Is a starving host tastier? Reproduction in fleas parasitizing food-limited rodents. – Funct. Ecol. 19: 625–631.
Kumura, H. et al. 2004. Screening of dairy yeast strains for probiotic applications. – J. Dairy Sci. 87: 4050–4056.
Lanot, R. et al. 2001. Postembryonic hematopoiesis in *Drosophila*. – Dev. Biol. 230: 243–257.
Lee, K. P. et al. 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. – Proc. R. Soc. Lond. B 273: 823–829.
Lee, K. P. et al. 2008. Dietary protein-quality influences melanization and immune function in an insect. – Funct. Ecol. 22: 1052–1061.
Li, P. et al. 2007. Amino acids and immune function. – Br. J. Nutr. 98: 237–252.
Lindsay, S. L. 1958. Food preferences of *Drosophila* larvae. – Am. Nat. 92: 279–285.
Logan, A. et al. 2005. The impact of host starvation on parasite development and population dynamics in an intestinal trypanosome parasite of bumble bees. – Parasitology 130: 637–642.
Macey, B. M. and Coyne, V. E. 2005. Improved growth rate and disease resistance in farmed *Haliotis midae* through probiotic treatment. – Aquaculture 245: 249–261.
Nagata, S. et al. 2006. Dietary sterol preference in the silkworm, *Bombyx mori*. – Biosci. Biotechnol. Biochem. 70: 3094–3098.
Noverr, M. C. and Huffnagle, G. B. 2004. Does the microbiota regulate immune responses outside the gut? – Trends Microbiol. 12: 562–568.
Ojala, K. et al. 2005. Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. – Evol. Ecol. Res. 7: 1153–1170.
Reid, G. et al. 2001. Can bacterial interference prevent infection? – Trends Microbiol. 9: 424–428.
Rivera, M. T. et al. 2003. Trace elements, innate immune response and parasites. – Clin. Chem. Lab. Med. 41: 1020–1025.
Rojas-Garcia, C. R. et al. 2008. Cell wall deficient *Saccharomyces cerevisiae* strains as microbial diet for *Artemia* larvae: protective effects against vibriosis and participation of phenoloxidase. – J. Exp. Mar. Biol. Ecol. 360: 1–8.
Seppälä, O. et al. 2008. Host condition as a constraint for parasite reproduction. – Oikos 117: 749–753.
Singer, M. S. et al. 2004. Disentangling food quality from resistance against parasitoids: diet choice by a generalist caterpillar. – Am. Nat. 164: 423–429.
Singer, M. S. et al. 2009. Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. – PLoS ONE 4: e4796.
Siva-Jothy, M. T. and Thompson, J. J. W. 2002. Short-term nutrient deprivation affects immune function. – Physiol. Entomol. 27: 206–212.
Starmer, W. T. 1981. A comparison of *Drosophila* habitats according to the physiological attributes of the associated yeast communities. – Evolution 35: 38–52.
Suwanchaichinda, C. and Paskewitz, S. M. 1998. Effects of larval nutrition, adult body size, and adult temperature on the ability of *Anopheles gambiae* (Diptera: Culicidae) to melanize Sephadex beads. – J. Med. Entomol. 35: 157–161.
Thompson, S. N. and Kavaliers, M. 1994. Physiological bases for parasite-induced alterations of host behaviour. – Parasitology 109 (Suppl.): 119–138.
Thompson, S. N. and Redak, R. A. 2005. Feeding behaviour and nutrient selection in an insect *Manduca sexta* L. and alterations induced by parasitism. – J. Comp. Physiol. A 191: 109–123.
Thompson, S. N. and Redak, R. A. 2008. Parasitism of an insect *Manduca sexta* L. alters feeding behaviour and nutrient utilization to influence developmental success of a parasitoid. – J. Comp. Physiol. B 178: 515–527.
Thompson, S. N. et al. 2005. Host nutrition determines blood nutrient composition and mediates parasite developmental success: *Manduca sexta* L. parasitized by *Cotesia congregata* (Say). – J. Exp. Biol. 208: 625–635.
van Alphen, J. J. M. and Janssen, A. R. M. 1982. Host selection by *Asobara tabida* Nees (Braconidae: Alysiinae): a larval parasitoid of fruit inhabiting *Drosophila* species. – Neth. J. Zool. 32: 194–214.

Vass, E. and Nappi, A. J. 1998. The effects of dietary yeast on the cellular immune response of *Drosophila melanogaster* against the larval parasitoid, *Leptopilina boulardi*. – J. Parasitol. 84: 870–872.
Waldbauer, G. P. and Friedman, S. 1991. Self-selection of optimal diets by insects. – Annu. Rev. Entomol. 36: 43–63.
Wertheim, B. et al. 2005. Genome-wide gene expression in response to parasitoid attack in *Drosophila*. – Gen. Biol. 6: R94.
Wilson, K. 2005. Evolutionary ecology of insect host-parasite interactions: an ecological immunology perspective. – In: Fellowes, M. et al. (eds), Insect evolutionary ecology. CABI Publishing, pp. 289–346.