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Body Size and Nutrition Intake Effects on Fecundity and Overwintering Success in *Anchomenus dorsalis* (Coleoptera: Carabidae)

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ABSTRACT. Structural body size and adult feeding conditions seem to be important determinants of fitness in income breeding species. However, little is known about the relative importance of structural body size and nutritional state on fecundity and winter survival in carabids. In this study, two separate experiments were performed. The effects of the structural body size of females (expressed as the length of the elytra and the width of the pronotum) and the effect of starvation on the fecundity of the ground beetle *Anchomenus dorsalis* (Pontoppidan, 1763) were investigated in the “fecundity experiment.” The influence of structural body size, feeding conditions (full, partial, or no feeding) before the winter, and behavior during the winter (burrowing into the substrate) on winter survival in *A. dorsalis* females were studied in the “overwintering experiment.” Egg production was positively influenced by both the structural body size of females and adult feeding. The effect of structural body size on the number of eggs laid outweighed the effect of feeding. However, the total fecundity (the number of eggs laid plus the number of mature eggs in ovaries) were more strongly affected by feeding in comparison to the structural body size of females. Interestingly, there was no significant effect of structural body size, feeding before winter, or behavior during winter on the survival of *A. dorsalis* females during the winter. However, our overwintering results could be affected by extreme weather conditions throughout experimental season and by the experimental design, which is discussed in detail.

Key Words: body mass, egg production, ground beetles, starvation, structural size

The body size of an organism is one of the most frequently studied characteristics in life sciences because it has a substantial impact on various aspects of the biology of a given organism, from physiology to ecology and evolution (Peters 1983). In a long-term macroevolutionary context, body size may have serious effects on speciation and extinction processes mediated by its effects on population density, resource exploitation, generation time, etc. (Martin and Palumbi 1993, Silva and Downing 1995). On the short-term ecological and microevolutionary timescale, body size is a crucial feature affecting individual fitness. Bigger individuals are able to prey upon larger prey (Christensen 1996) and generally have enhanced longevity (Blanckenhorn et al. 2007), winter survival (Kovacs and Goodisman 2012), mating success (Peixoto and Benson 2008), and fecundity (Honěk 1993). Nonetheless, there are also some disadvantages of large adult body size that stem from increased juvenile mortality caused, e.g., by enhanced predation risk linked to prolonged development or by enhanced physiological stress linked to increased growth rate (Blanckenhorn 2000).

When investigating the influence of body size on fitness, methodological problems could stem from the fact that body size could be represented by several disparate measures such as body mass or structural body size (Strobbe and Stoks 2004). In insects, plasticity in structural body size (e.g., leg length, elytron length, head width, or wingspan) is caused by environmental conditions experienced during development, and structural body size does not change over the adult lifetime (Irwin and Lee 2000). In contrast, body mass is highly variable throughout the adult lifetime depending on the environmental conditions experienced as adults (Chaabane et al. 1997, Bommarco 1998, Knapp and Knappová 2013). Deciding which measure to use in a particular case depends on the biology of the species investigated and on the particular research question. Some species, known as capital breeders, acquire the entire energy budget designated for reproduction during development, whereas others, known as income breeders, acquire energy for reproduction even during adulthood (Wessels et al. 2010, Pelisson et al. 2012).

In capital breeders, structural body size seems to be the most meaningful measure of body size related to fecundity, longevity, winter survival, etc. In income breeders, body mass is a commonly used measure for the same purposes (e.g., Zanutto et al. 2002, Piironen et al. 2011). Several studies attempted to investigate the relative importance of the environmental conditions experienced as juveniles and adults for income breeders via measuring the effects on structural body size and body mass simultaneously (Li et al. 2009, Wessels et al. 2010). Variation in adult body mass is tightly linked to nutrition intake in carabids (Chaabane et al. 1997, Bommarco 1998). However, the relative importance of juvenile conditions represented by structural body size and the nutritional conditions experienced by adults on fecundity has been rarely investigated in carabid beetles (but see Ernsting et al. 1992).

To achieve high realized fitness, it is crucial to survive until reproductive maturity. In temperate climates, winter is a period of increased risk for insect survival, whereas winter mortality may reach 90% in some species (Leather et al. 1995). Insects have limited ability to regulate their internal temperature, and therefore, an ambient temperature that differs substantially from their physiological optimum causes physiological stress (Bale and Hayward 2010). To cope with harsh winter conditions, insects had to evolve various physiological and behavioral adaptations such as synthesis of cryoprotectants, accumulation of energy reserves prior to winter, or finding a suitable overwintering microhabitat that buffers extreme minimum temperatures (Luff 1966, Ernsting et al. 1992, Bale and Hayward 2010). Large structural body size and large body mass seem to enhance winter survival in insects (Piironen et al. 2011, Kovacs and Goodisman 2012). However, the relative importance of particular components of body size (structural size and mass) and behavior on winter survival has been minimally investigated.

In this study, we investigated the effects of female structural body size and adult nutritional conditions on fecundity in the ground beetle *Anchomenus dorsalis* (fecundity experiment). We also investigated the

effects of structural body size, adult prewinter nutritional conditions, and winter behavior (digging into the substrate) on winter survival of *A. dorsalis* females (overwintering experiment). We hypothesize the following: 1) fecundity is affected not only by adult feeding, which is frequently reported for carabids, but also by female structural body size and 2) winter survival is affected by structural body size, feeding prior to the winter, and overwintering behavior (burying behavior).

Materials and Methods

Study Species. *Anchomenus dorsalis* (Pontoppidan, 1763) is a common Eurasian carabid beetle (Coleoptera: Carabidae). Adults are medium-sized beetles that are 5.6–7.7 mm long with considerable sexual size dimorphism, whereas females are generally larger than males (Hůrka 1996, Knapp 2012). In the Czech Republic, this species can be found in drier open areas such as arable fields, meadows, dry grasslands (steppes), and gardens (Hůrka 1996, Stanovský and Pulpán 2006). This species is a typical spring breeder that overwinters as an adult, breeds during May and June, and larva grows in the early summer (Fazekas et al. 1999, Holland 2002). Populations of *A. dorsalis* inhabiting arable fields migrate to neighboring noncrop habitats in autumn for overwintering and in the spring return to arable fields (Holland 2002). In Central Europe, *A. dorsalis* specimens frequently overwinter buried in the top soil layer (~10–20 cm under the surface in small cavities under stones, etc.; Michal Knapp, personal observation). Beetles do not seem to enter winter diapause (just quiescence), given that they started to move a few minutes after being heated to room temperature (20°C). *A. dorsalis* prey upon small arthropods, e.g., aphids, and are considered a beneficial species with the potential for biocontrol of agricultural pests (Chiverton and Sotherton 1991).

Overwintering Experiment. The aim of the overwintering experiment was to assess the effects of structural body size, nutrition income before winter, and behavior during winter (burying in sand) on the overwintering success of *A. dorsalis* females. The adult beetles used in this experiment were collected in September 2011 at the edge of arable fields located near Prague–Suchdol, the Czech Republic. Specimens were subsequently transferred to the Crop Research Institute in Prague–Ruzyně, where the following experiment was carried out. Beetles were sexed, and all males were released back to nature. In total, 180 randomly selected females were assigned to one of the following three treatments: 1) full feeding, 2) partial feeding, and 3) no feeding. In each treatment, specimen size ranged from small to large, and there were no differences in the mean structural body size of beetles among particular treatments (principal component analysis [PCA] ax1 scores; ANOVA: $F_{2,128} = 0.216$, $P = 0.806$).

Feeding treatments were run for 6 wk. Beetles were reared in groups in large glass Petri dishes (25 cm in diameter; three dishes per treatment) filled with sandy substrate. All specimens were supplied with water ad libitum. Beetles in the full feeding treatment were fed mealworms (*Tenebrio molitor* L. larvae) cut to pieces for entire period, beetles in the partial feeding treatment were fed mealworms for 3 wk, and beetles in the no feeding treatment were not fed at all. Fresh mealworms were added twice a week in excess, and at the same time, all remaining old food were removed. Beetles were kept under field conditions (Petri dishes were placed in grassland next to the site barrack), so the environmental conditions (e.g., photoperiod and course of temperature) fit the natural ones. There were significant differences in the mean body mass between particular feeding treatments (ANOVA: $F_{2,128} = 7.56$, $P = 0.001$) based on fresh body mass measurements of beetles after 6 wk of treatment application. The mean fresh body mass was 11.5 mg for starved beetles, 13.3 mg for partially fed beetles, and 14.4 mg for fully fed beetles.

After 6 wk, at the end of October 2011, beetles were individually placed into perforated plastic pots and relocated to the overwintering site. Plastic cups (0.2 liter in volume, 7 cm in diameter, and 9 cm in height) were filled with 3 cm of sandy substrate and covered with fine mesh to prevent the beetles from escaping. No food was added to the

pots. The overwintering site was located in the Crop Research Institute in Prague–Ruzyně and composed of a grassy boundary situated under pear trees (just few meters away from the site barrack). This area is an overwintering site for the natural population of *A. dorsalis* (personal observation). All pots with beetles were placed close to each other, and after 2 wk, they were covered by plastic foil to prevent extreme flooding. The sandy substrate within the pots remained wet until the end of the experiment, so the beetles did not suffer from low humidity. One pot containing a datalogger was added to those with beetles to measure the temperature over the course of the winter. Temperatures were recorded every 2 h (see Appendix 1).

Overwintering beetles were checked once a month from November 2011 to March 2012 to record their behavior. Beetles on the surface all the times were classified as “unburied,” and these buried in the sand in at least one census were classified as “buried.” At the end of March 2012, all overwintered beetles were taken to the laboratory, and their survival (yes or no) was recorded after 24 h spent at 20°C in laboratory conditions. All live beetles were killed by freezing. In early April, the structural body size of all beetles (including those that died during the winter) was measured using a digital caliper to a precision of 0.01 mm. For each specimen, the elytron length and pronotum width were recorded. Some specimens most likely escaped from the pots during the overwintering experiment (no bodies were found in some pots), so the final dataset consists of 130 beetles.

Fecundity Experiment. The aim of the fecundity experiment was to assess the effects of the structural body size of females, nutrition income, and structural body size of males (mates) on the fecundity of *A. dorsalis* females. Beetles used in the fecundity experiment were collected at the beginning of April 2012 on the edge of two fields near Prague–Suchdol, the Czech Republic. In total, >400 individuals were collected. We specifically attempted to collect small and large specimens and to omit midsized ones. Subsequently, beetles were transferred to the Crop Research Institute, where the fecundity experiment was carried out.

All beetles were sexed, and the 50 smallest and 50 largest females (assessed visually) and 100 males selected at random were chosen for the fecundity experiment. Methodological restrictions prevented us from precisely measuring the beetles before experiment termination (live beetles are not easy to measure without injuring them). At the beginning of the experiment, one female and one randomly selected male were placed together in a plastic pot. Pots were 7 cm in diameter, and the substrate was made of a mixture of plaster of Paris and charcoal (to prevent mildew). Using a solid substrate instead of a sandy one enhances the probability of successfully finding eggs. Beetles were reared in the underground laboratory at a constant temperature of 19°C, relative humidity ~60%, and photoperiod set to 16:8 (L:D) h (light was provided by two fluorescent tubes). All specimens were supplied with water ad libitum over the course of the experiment using modified Eppendorf tubes with the original cap replaced by a piece of cotton wool. In the first week of the experiment, all pairs were fed (mealworms and dog biscuits) ad libitum. From the second week of the experiment until its termination (after 10 wk), the beetles were divided into the following two treatments: 1) fed and 2) starving. Each treatment included 50 pairs, and large and small females were divided equally between the treatments. Individuals in the fed treatment were supplied with food (mealworms) three times a week. Beetles in the starving treatment were not fed at all and were supplied with water only.

At the same time that food was supplied in the fed treatment (i.e., three times a week), all plastic pots (for both fed and starving treatments) were checked for eggs. Egg numbers were recorded, and the eggs were removed. At the end of the fecundity experiment (late June 2012), all beetles included were killed by freezing and stored in a freezer at –20°C for future processing. Subsequently, all specimens were measured for structural body size (elytron length and pronotum width) using a digital caliper to 0.01 mm. Moreover, females were dissected, and all mature eggs in their ovaries were counted using an

Olympus SZX7 stereomicroscope. Some beetles included in the fecundity experiment died during the course of the experiment. We decided to exclude all pairs in which either the male or female died from the analyses, resulting in a final dataset consisting of 81 pairs (40 pairs in the fed treatment and 41 pairs in the starving treatment).

Statistical Analyses. To obtain a single variable quantifying the structural body size of the beetles, PCA based on the measurements of elytron length and pronotum width was conducted. The structural body size of each particular specimen was expressed as its score on the first axis in the PCA. Separate analyses (PCA) were conducted for datasets originating from each experiment (overwintering and fecundity experiment). Ordination analyses were performed in Canoco for Windows 4.5 software (ter Braak and Smilauer 2002).

To analyze the effects of structural body size, feeding, and behavior on the winter survival of *A. dorsalis* females, a generalized linear model with binomial errors distribution (GLM-b) was employed. The full model, including all main effects (feeding treatment, structural body size, and burying behavior) and all possible interactions, was constructed followed by a backward selection procedure based on deletion tests (χ^2 tests).

To analyze the effects of the structural body size of the female, feeding, and the structural body size of male on the fecundity of *A. dorsalis* females, a generalized linear model with negative binomial errors distribution (GLM-nb) was employed. To control for possible effects of male body size on fecundity, the structural body size of males was also analyzed. The independent variables included in each model were the structural body size of the female, feeding treatment, interaction of body size of female \times feeding treatment, and structural body size of the male. The significance of particular terms was assessed based on χ^2 tests. Models were constructed separately for the following three response variables: 1) number of eggs laid during the 10-wk period, 2) number of eggs in ovaries at the end of the experiment, and 3) total number of eggs produced (sum of the two preceding variables). To assess the relative importance of feeding and the structural body size of females on fecundity in *A. dorsalis*, a variance partitioning procedure (based on GLM-nb) was employed. All analyses were performed with R 2.15.2 software (R Development Core Team 2012).

Results

The survival rate in the overwintering experiment was generally low. Only 17 out of 130 females (i.e., 13%) were alive at the end of the experiment in March 2012 (5 out of 48 starving beetles, 4 out of 37 partially fed beetles, and 8 out of 45 fully fed beetles survived). The survival rate was not significantly affected by structural body size (GLM-b: $\chi^2_1 = 0.41$, $P = 0.52$) or feeding treatment (GLM-b: $\chi^2_2 = 1.37$, $P = 0.50$). Specimens that spent the entire winter period on the surface had two times lower probability of survival (6 out of 67 survived) in comparison to the beetles that burrowed into the substrate (11 out of 63 survived). However, this difference was not statistically significant (GLM-b: $\chi^2_1 = 2.09$, $P = 0.15$). There were no significant interaction effects (GLM-b: all $P > 0.3$).

The number of eggs laid was positively affected by higher structural body sizes of females (GLM-nb: $\chi^2_1 = 11.13$, $P < 0.001$; Fig. 1A) and feeding (GLM-nb: $\chi^2_1 = 8.09$, $P = 0.004$; Fig. 2A). The structural body size of males (GLM-nb: $\chi^2_1 = 0.78$, $P = 0.38$) and interaction between the structural body size of females and feeding treatment (GLM-nb: $\chi^2_1 = 0.73$, $P = 0.39$) did not affect the number of eggs laid. The number of mature eggs in ovaries at the end of the fecundity experiment was significantly affected by feeding treatment (GLM-nb: $\chi^2_1 = 133.02$, $P < 0.001$); starved females had no mature eggs in their ovaries. The number of mature eggs was positively affected by the structural body size of females (GLM-nb: $\chi^2_1 = 4.12$, $P = 0.042$), and there was also a significant interaction between the structural body size of females and feeding treatment (GLM-nb: $\chi^2_1 = 5.33$, $P = 0.021$). The structural body size of males (GLM-nb: $\chi^2_1 = 3.17$, $P = 0.075$) had no effect on the number of mature eggs in ovaries at the end of the fecundity

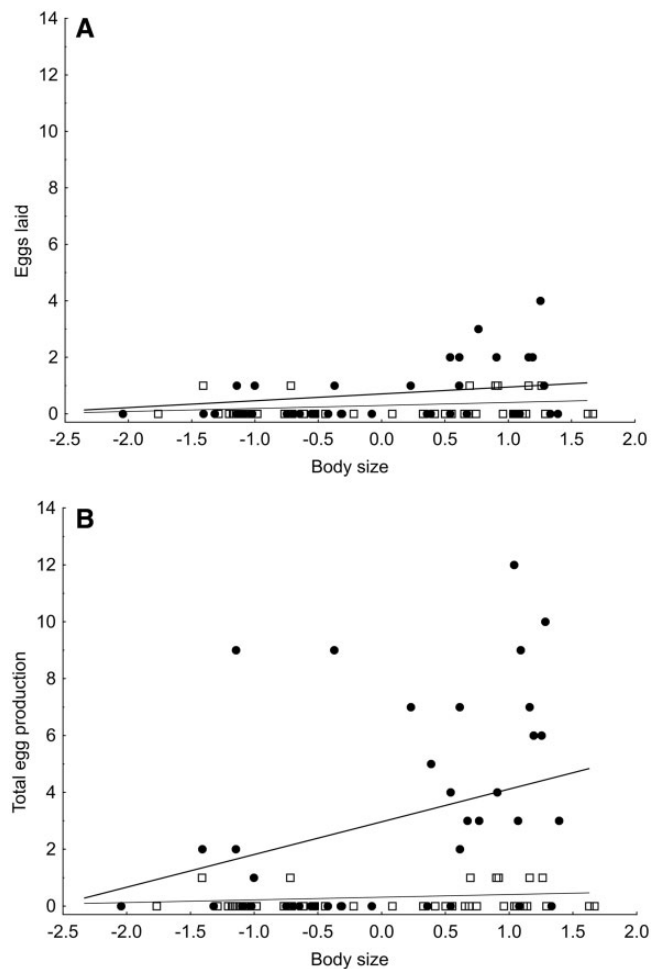


Fig. 1. The effect of structural body size on fecundity of *A. dorsalis* expressed as follows: (A) number of eggs laid during the 10-wk period and (B) total egg production (number of eggs laid plus the number of mature eggs in ovaries at the end of the experiment). Full circles represent fed females (bold line), and open squares represent starved females (thin line). Body size is expressed as the score on first axis of PCA based on elytron length and pronotum width.

experiment. Total egg production was positively affected by feeding treatment (GLM-nb: $\chi^2_1 = 47.84$, $P < 0.001$; Fig. 2B) as well as by the structural body size of females (GLM-nb: $\chi^2_1 = 8.49$, $P = 0.004$, Fig. 1B). There was significant interaction between the structural body size of females and feeding treatment (GLM-nb: $\chi^2_1 = 4.14$, $P = 0.042$). The structural body size of males (GLM-nb: $\chi^2_1 = 1.57$, $P = 0.210$) had no effect on total egg production. The structural body size of females was a slightly more important determinant of the number of eggs laid than the feeding treatment (Fig. 3A). The opposite pattern was apparent for total egg production, which was determined mainly by feeding treatment (Fig. 3B).

Discussion

The lack of a link between winter survival of *A. dorsalis* and any trait investigated in this study (i.e., structural body size, prewinter feeding conditions, and overwintering behavior) is surprising because the winter survival of various insect species is affected by these traits (Leather et al. 1995, Piironen et al. 2011, Kovacs and Goodisman 2012). One possible explanation of such an unexpected result could be the extremely hard winter that occurred during the experimental season. The minimum daily temperature on one above-ground meter reached -20°C for a 14-day period in February 2012, which is an extraordinary

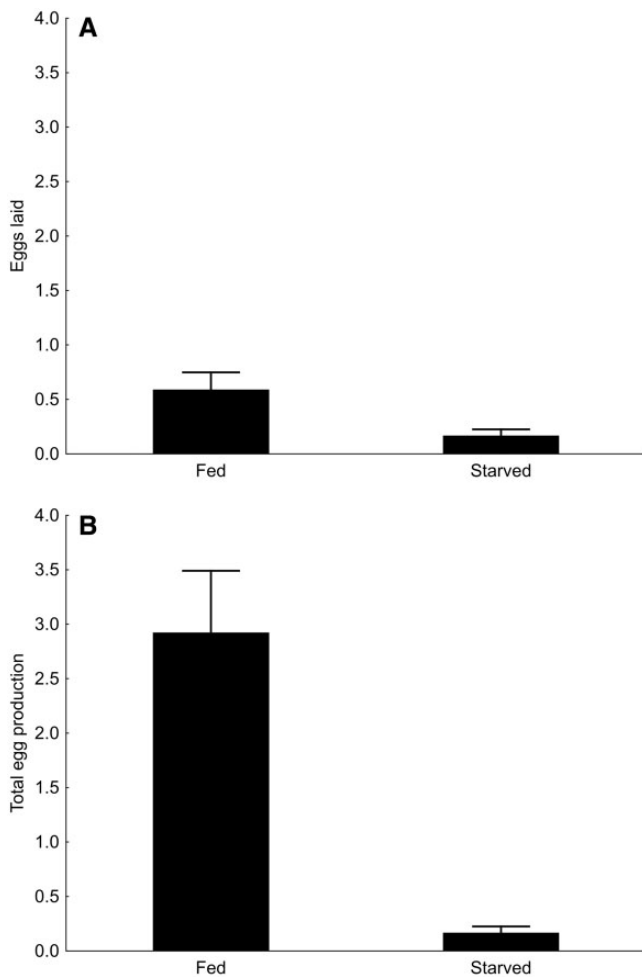


Fig. 2. The effect of feeding on fecundity of *A. dorsalis* expressed as follows: (A) number of eggs laid during the 10-wk period and (B) total egg production (number of eggs laid plus the number of mature eggs in ovaries at the end of the experiment). Values are the means \pm SE.

event in Prague (Czech Hydrometeorological Institute 2013). In the same period, the temperatures recorded by the datalogger placed under snow-cover inside a plastic cup in the same temperature conditions experienced overwintering beetles were constantly below 0°C (see Appendix 1). The low winter survival observed (only 13%) is most likely a result of these extremely low temperatures. The survival rates reported for carabids with similar biology, i.e., temperate spring breeders wintering as adults, are commonly much higher, reaching up to 90% (Van Dijk 1994, Knapp and Saska 2012). Low winter mortality (ca. 4%) was also recorded for *A. dorsalis* by Riedel and Steenberg (1998), although the last census in their study occurred in February, and it is probable that additional mortality occurred before the end of overwintering in early April.

The usage of plastic cups for the overwintering experiment could be an additional cause of the low winter survival observed. *A. dorsalis* in Central Europe frequently overwinter buried in the soil, ~10–20 cm under surface, frequently in small groups of five to ten individuals (M.K., personal observation). Unfortunately, the depth of the substrate in cups used for overwintering in this study was limited to only 3 cm. Beetles buried at greater depth are better protected against temperature extremes (both heat and cold), and such thermal buffering could result in enhanced winter survival (Bale and Hayward 2010). The relevance of burying behavior during the winter season is indicated also by this study because beetles that were buried (even in only 3 cm of substrate) had a doubled probability of winter survival, although the statistical test

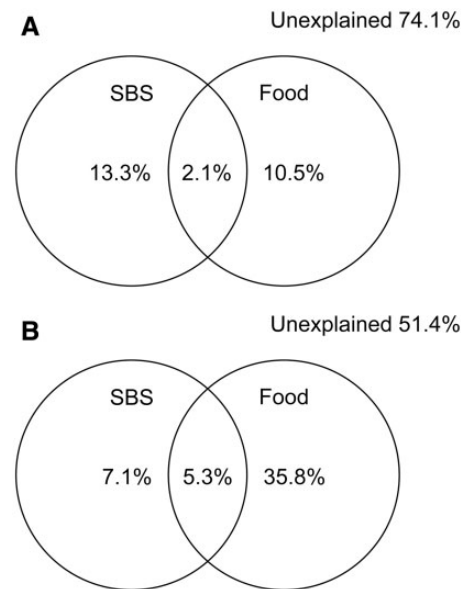


Fig. 3. The relative importance of structural body size and feeding on fecundity in *A. dorsalis* females. Variance partitioning based on generalized linear models with negative binomial errors distribution was employed. Percentage of variance explained by structural body size (SBS), feeding (food), and shared variance are shown for (A) the number of eggs laid during the 10-wk period and (B) the total egg production (number of eggs laid plus the number of mature eggs in ovaries at the end of the experiment).

was insignificant. As mentioned above, *A. dorsalis* frequently occurs in aggregations in nature, whereas beetles were placed in pots individually in our study. Zetto-Brandmayr et al. (2006) proposed the hypothesis that the aggregation behavior of *A. dorsalis* (during vegetation season) enhances their resistance against predators. Unfortunately, it is not clear whether this behavior taking place during the winter season serves as protection against winter-active predators or whether it enables beetles to better cope with abiotic conditions.

It is surprising that no significant relationship was found between winter mortality and food supply in the prewinter period. However, our results could be substantially affected by the low survival rate, resulting in low power of the applied statistical test. Several studies documented that spring-breeding carabids (overwintering as adults) cumulate energy reserves through the late summer and autumn, and these reserves are subsequently almost completely exhausted through the winter (Ernsting et al. 1992, Van Dijk 1994). A similar pattern in seasonal variance in dry weight and fat content was also observed for the Central European population of *A. dorsalis* (M.K., unpublished data). However, the effect of the nutritional condition on winter survival in carabid beetles seems to vary seasonally (Van Dijk 1994), most likely due to the season-specific course of winter temperatures. There are indications that mild winters (with higher temperatures) cause mortality due to exhaustion of energy reserves, whereas hard winters (with low temperatures) cause mortality due to chill injuries (Leather et al. 1995, Petersen et al. 1996, Irwin and Lee 2000). This could be the cause of the lack of effect of feeding treatment on winter mortality reported in this study. Moreover, building fat reserves depends on the quantity and the quality of ingested food, i.e., the content of proteins, carbohydrates, and other important substances (Arrese and Soulagés 2010). It is possible that the beetles provided with a single food type (mealworms in this study) are not in an optimal nutritional condition, analogous to the decrease in fecundity due to unbalanced food composition reported for *A. dorsalis* by Jensen et al. (2012). The alternative explanation is that all beetles (including those from the “no feeding treatment”) used in our study had sufficient energy reserves. The beetles were collected in

September, i.e., 1 or 2 mo after their eclosion, so they had an opportunity to build energy reserves prior to the start of our experiment.

Although the larger body size of an individual seems to be generally linked to higher winter survival, it may not be a universal rule. The relative efficiency hypothesis predicts that larger individuals are favored in stressful winter conditions due to a proportionally lower metabolism rate (Glazier 2005). In contrast, the absolute energy demand hypothesis predicts that larger individuals are disadvantaged by their higher mass due to their higher total energy expenditure (Ismail et al. 2012). Mixed results supporting both hypotheses are reported for insects (Blanckenhorn et al. 2007, Piironen et al. 2011, Ismail et al. 2012). Thus, our results indicating no relationship between structural body size and winter survival in *A. dorsalis* are plausible. However, future studies in *A. dorsalis* are needed to confirm our results under weather conditions and an experimental setup resulting in lower winter mortality than that observed in this study.

Our results demonstrate that egg production in *A. dorsalis* was positively affected by both the structural body size of females and the feeding treatment experienced as adults. To our knowledge, the effect of structural body size on fecundity in carabids has rarely been studied. Attention was mainly paid to effects of nutrition intake because carabids eclose with almost no energy reserves (fat reserves), and these reserves are necessary for egg production (Chaabane et al. 1997, Bommarco 1998). Structural body size had a substantial effect on the number of eggs laid during our fecundity experiment, whereas it had a much lower effect on the number of eggs in the ovaries of dissected females. One possible explanation is that the eggs laid were made with energy reserves accumulated by adults in the field prior to collection and laid during the first week of the fecundity experiment (in a time when all females had access to food), whereas the eggs in ovaries were made from sources acquired in the course of the fecundity experiment. Starved females actually had no mature eggs in their ovaries after 9 wk of starvation, which could be caused by ceasing egg maturation or even by oosorption.

Structural body size in adult insects can be determined both genetically and by developmental plasticity resulting from the environmental conditions experienced during larval development (e.g., ambient temperature or diet quality and quantity; Bommarco 1998, Blanckenhorn 2000). Beetles used in this study were collected as adults, so we are not able to determine the cause (genetic vs. developmental plasticity) of their variation in structural body size. To our knowledge, there are no studies investigating the underlying causes of intraspecific variation in structural body size in *A. dorsalis*. However, both genetically based intraspecific differences in structural body size (Tsuchiya et al. 2012) and phenotypic plastic reaction to food quantity (Bommarco 1998) are known to exist in carabids. Thus, the larval environment and genetic predispositions may affect fecundity in adult *A. dorsalis*. It has been shown that juvenile development or genetic predispositions affect adult fecundity in some carabids, e.g., *Poecilus cupreus*. The effect of locality on fecundity in *P. cupreus* was not fully rescued by adult feeding (beetles originating from various sites most likely differed in the larval conditions experienced or in genetic predispositions; Bommarco 1998).

The substantial effect of feeding conditions on fecundity in *A. dorsalis* is in accord with previous studies performed on various carabid species (Thiele 1977, Wallin et al. 1992, Bilde and Toft 1994, Lövei and Sunderland 1996, Holland 2002, Jensen et al. 2012). The first priority of the adults is to meet the energy demands necessary for maintenance (survival), and the surplus is used for reproduction (Van Dijk 1994, Lövei and Sunderland 1996). It is important to note that the fed beetles investigated in this study produced relatively few eggs—~3 eggs/female—which could be due to suboptimal (imbalanced) diet. Beetles were only provided mealworms (and, in the first week, dog biscuits as well). There is evidence for *A. dorsalis* that an imbalanced diet (e.g., ratio of proteins, lipids, and micronutrients) reduces egg production (Bilde and Toft 1994, Jensen et al. 2012). The egg production

recorded in this study is in between the values reported for *A. dorsalis* fed earthworms (0.5 eggs/female) and for beetles provided with a variety of food types (earthworms, aphids and fruit flies; 5.6 eggs/female; Bilde and Toft 1994). This could indicate that mealworms (the larvae of darkling beetles) are not the most optimal diet for *A. dorsalis*.

In conclusion, no effects of structural body size and feeding prior to overwintering on winter survival were observed in this study. However, there was extremely high winter mortality, which most likely affected the reported results. Thus, future studies investigating the effects of body size and body condition in *A. dorsalis* are needed. Fecundity in *A. dorsalis* was significantly affected by both female structural body size and feeding differed for the number of eggs laid (during the 10 wk of the experiment) and total egg production (eggs laid plus mature eggs in ovaries at the end of the experiment). The number of eggs laid was more significantly affected by female structural body size, whereas total egg production was more substantially affected by feeding treatment. To our knowledge, the significance of structural body size for egg production in carabids has not been reported to date. Variance in structural body size can be caused either by the environmental conditions experienced during larval growth (i.e., developmental plasticity) or by genetic predispositions. Unfortunately, this study is not able to distinguish which of these factors affecting structural body size is a more important determinant of adult egg production, and future studies investigating this topic are needed.

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References Cited

- Arrese, E. L., and J. L. Soulages. 2010. Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* 55: 207–225.
- Bale, J. S., and S.A.L. Hayward. 2010. Insect overwintering in a changing climate. *J. Exp. Biol.* 213: 980–994.
- Bilde, T., and S. Toft. 1994. Prey preference and egg production of the carabid beetle *Agonum dorsale*. *Entomologia Experimentalis et Applicata* 73: 151–156.
- Blanckenhorn, W. U. 2000. The evolution of body size: what keeps organisms small? *Quart. Rev. Biol.* 75: 385–407.
- Blanckenhorn, W. U., J. Fanti, and C. Reim. 2007. Size-dependent energy reserves, energy utilization and longevity in the yellow dung fly. *Physiol. Entomol.* 32: 372–381.
- Bommarco, R. 1998. Stage sensitivity to food limitation for a generalist arthropod predator, *Pterostichus cupreus* (Coleoptera: Carabidae). *Environ. Entomol.* 27: 864–869.
- Chaabane, K., M. Loreau, and G. Josens. 1997. Growth and egg production in *Abax ater* (Coleoptera, Carabidae). *Pedobiologia* 41: 385–396.
- Chiverton, P. A., and N. W. Sotherton. 1991. The effects of beneficial arthropods of the exclusion of herbicides from cereal crop edges. *J. Appl. Ecol.* 28: 1027–1039.
- Czech Hydrometeorological Institute. 2013. Czech Hydrometeorological Institute, Prague. (www.chmi.cz).
- Christensen, B. 1996. Predator foraging capabilities and prey antipredator behaviours: Pre- versus postcapture constraints on size-dependent predator-prey interactions. *Oikos* 76: 368–380.
- Ernsting, G., J. A. Isaaks, M. P. Berg. 1992. Life-cycle and food availability indexes in *Notiophilus biguttatus* (Coleoptera, Carabidae). *Ecol. Entomol.* 17: 33–42.
- Fazekas J. P., F. Kadar, M. Sarospataki, and G. L. Lovei. 1999. Seasonal activity, and reproduction in the spring breeding ground beetle species *Agonum dorsale* and *Brachinus explodens* in Hungary (Coleoptera: Carabidae). *Entomologia Generalis* 23: 259–269.
- Glazier, D. S. 2005. Beyond the '3/4 power law': variation in the intra and inter-specific scaling of metabolic rate in animals. *Biol. Rev.* 80: 611–662.
- Holland, J. 2002. The agroecology of carabid beetles. Intercept Limited, Andover, UK.

- Honěk A. 1993.** Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* 66: 483–492.
- Hůrka, K. 1996.** Carabidae of the Czech and Slovak Republics. Kabourek, Zlín, CZE.
- Irwin, J. T., and R. E. Lee. 2000.** Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *J. Insect Physiol.* 46: 655–661.
- Ismail, M., P. Vernon, T. Hance, J.S. Pierre, and J. Van Baaren. 2012.** What are the possible benefits of small size for energy-constrained ectotherms in cold stress conditions? *Oikos* 121: 2072–2080.
- Jensen, K., D. Mayntz, S. Toft, F. J. Clissold, J. Hunt, D. Raubenheimer, and S. J. Simpson. 2012.** Optimal foraging for specific nutrients in predatory beetles. *Proc. R. Soc. B Biol. Sci.* 279: 2212–2218.
- Knapp, M. 2012.** Preservative fluid and storage conditions alter body mass estimation in a terrestrial insect. *Entomologia Experimentalis et Applicata* 143: 185–190.
- Knapp, M., and J. Knappová. 2013.** Measurement of body condition in a common carabid beetle, *Poecilus cupreus*: a comparison of fresh weight, dry weight, and fat content. *J. Insect Sci.* 13: 6.
- Knapp, M., and P. Saska. 2012.** The effects of habitat, density, gender and duration on overwintering success in *Bembidion lampros* (Coleoptera: Carabidae). *J. Appl. Entomol.* 136: 225–233.
- Kovacs, J. L., and M. A. Goodisman. 2012.** Effects of size, shape, genotype, and mating status on queen overwintering survival in the social wasp *Vespula maculifrons*. *Environ. Entomol.* 41: 1612–1620.
- Leather, S. R., K. F. Walters, and J. S. Bale. 1995.** The ecology of insect overwintering. Cambridge University Press, Cambridge.
- Li, H., S. Toepfer, and U. Kuhlmann. 2009.** Relationship between phenotypic traits and selected fitness components of *Diabrotica virgifera virgifera*. *Entomologia Experimentalis et Applicata* 131: 254–263.
- Lovei, G. L., and K. D. Sunderland. 1996.** Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annu. Rev. Entomol.* 41: 231–256.
- Luff, M. L. 1966.** Cold hardiness of some beetles living in grass tussocks. *Entomologia Experimentalis et Applicata* 9: 191–199.
- Martin, A. P., S. R. Palumbi. 1993.** Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl Acad. Sci. USA.* 90: 4087–4091.
- Peixoto, P.E.C., and W. W. Benson. 2008.** Body mass and not wing length predicts territorial success in a tropical satyrine butterfly. *Ethology* 114: 1069–1077.
- Pelisson, P. F., M. C. Bel-Venner, B. Rey, L. Burgevin, F. Martineau, F. Fourel, S. Venner. 2012.** Contrasted breeding strategies in four sympatric sibling insect species: when a proovigenic and capital breeder copes with a stochastic environment. *Funct. Ecol.* 26: 198–206.
- Peters, R. H. 1983.** The ecological implications of body size. Cambridge University Press, Cambridge, UK.
- Petersen, M. K., B. Ekbom, and H. P. Ravn. 1996.** Temperature dependent winter survival of *Bembidion lampros* and *Tachyporus hypnorum*. *J. Insect Physiol.* 42: 997–1005.
- Piironen, S., T. Ketola, A. Lyytinen, and L. Lindstrom. 2011.** Energy use, diapause behaviour and northern range expansion potential in the invasive Colorado potato beetle. *Funct. Ecol.* 25: 527–536.
- R Development Core Team. 2012.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (<http://www.R-project.org/>).
- Riedel W., and T. Steenberg. 1998.** Adult polyphagous coleopterans overwintering in cereal boundaries: winter mortality and susceptibility to the entomopathogenic fungus *Beauveria bassiana*. *BioControl* 43: 175–188.
- Silva, M., and J. A. Downing. 1995.** The allometric scaling of density and body mass: a nonlinear relationship for terrestrial mammals. *Am. Nat.* 145: 704–727.
- Stanovský, J., and J. Pulpán. 2006.** Carabidae beetles of Silesia (North Moravia). Beskydy Museum, Frýdek-Místek, CZE.
- Strobbe, F., and R. Stoks. 2004.** Life history reaction norms to time constraints in a damselfly: differential effects on size and mass. *Biol. J. Linn. Soc.* 83: 187–196.
- ter Braak, C. J. F., and P. Šmilauer. 2002.** CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5), Microcomputer Power, Ithaca, USA.
- Thiele, H. U. 1977.** Carabid beetles in their environments: a study on habitat selection by adaptations in physiology and behaviour. Springer, Berlin, GER.
- Tsuchiya, Y., Y. Takami, Y. Okuzaki, and T. Sota. 2012.** Genetic differences and phenotypic plasticity in body size between high- and low-altitude populations of the ground beetle *Carabus tosanus*. *J. Evol. Biol.* 25: 1835–1842.
- Van Dijk, T. S. 1994.** On the relationship between food, reproduction and survival of 2 carabid beetles—*Calathus melanocephalus* and *Pterostichus versicolor*. *Ecol. Entomol.* 19: 263–270.
- Wallin, H., P. A. Chiverton, B. S. Ekbom, and A. Borg. 1992.** Diet, fecundity and egg size in some polyphagous predatory carabid beetles. *Entomologia Experimentalis et Applicata* 65: 129–140.
- Wessels, F. J., D. C. Jordan, and D. A. Hahn. 2010.** Allocation from capital and income sources to reproduction shift from first to second clutch in the flesh fly, *Sarcophaga crassipalpis*. *J. Insect Physiol.* 56: 1269.
- Zanuncio, J. C., A. J. Molina-Rugama, G. P. Santos, and F.D.S. Ramalho. 2002.** Effect of body weight on fecundity and longevity of the stinkbug predator *Podisus rostralis*. *Pesquisa Agropecuária Brasileira* 37: 1225–1230.
- Zetto-Brandmayr, T., T. Bonacci, A. Massolo, and P. Brandmayr. 2006.** What is going on between aposematic carabid beetles? The case of *Anchomenus dorsalis* (Pontoppidan 1763) and *Brachinus sclopeta* (Fabricius 1792) (Coleoptera Carabidae). *Ethol. Ecol. Evol.* 18: 335–348.

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Appendix 1

The course of temperatures experienced by *A. dorsalis* during the overwintering experiment.

