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Evidence of genetic basis of zoophagy and nymphal developmental time in isogroup lines of the zoophytophagous mullein bug, *Campylomma verbasci*

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Abstract In zoophytophagous predators genetically determined efficiency to exploit either animal or plant resources could lead to diet specialization, and a trade-off between fitness performances on animal and plant diet. Such a trade-off would have important consequences on the efficiency of zoophytophagous species as biocontrol agents. We estimated the genetic basis for zoophagy and nymphal development length and the genetic correlation between these two traits, in the mullein bug, *Campylomma verbasci* (Meyer) (Hemiptera: Miridae). In the laboratory, we counted the number of spider mites and aphids killed in 24 h by *C. verbasci* from 12 isogroup lines, and the nymphal development length under a plant and a mixed diet. Among-line variance in the level of zoophagy on both prey was significant and positively correlated. Diet had no significant effect on the mean nymphal development length, but lines differed in nymphal development regardless of the diet. Our results reveal genetic differences in foraging efficiency on prey, which suggest that some genotypes in population of the zoophytophagous mullein bug could provide more benefits in apple orchards.

Keywords Zoophytophagous predator · True omnivory · Genetic differences · Biological control · Genetic improvement · Hemiptera

Introduction

In many organisms specializing on a given resource could decrease the efficiency of foraging on alternative resources (Bolnick et al. 2003). Individuals may exploit different subsets of the population's food resources, resulting in diet variation among individuals in a population (Bolnick et al. 2003). As a consequence individuals within a population may not be considered as ecologically equivalent (Bolnick et al. 2003, 2011; Sih et al. 2012). This is the case of zoophytophagous predators that feed preferentially on prey, but can substitute or complement prey with plant resources (Coll and Guershon 2002; Arno et al. 2010). Plant and prey resources vary in space and time for a wide range of characteristics (e.g. chemical composition, nutritional value, defence strategies, distribution, availability) (Cohen 1996; Coll and Guershon 2002). Such variation may generate a trade-off between foraging efficiency on different types of resource.

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Trade-offs can occur in basically all the aspects of foraging behaviour such as resource recognition, capture, handling and digestion (Coll and Guershon 2002; Cohen 1996; Boyd et al. 2002; Roitberg et al. 2005). Consequently, instead of being composed of highly plastic genotypes that can easily switch from a zoophagous to a phytophagous diet, zoophytopagous populations may be composed of a mix of specialised genotypes that rely mostly on prey ("zoophagous strategy"), or on plant resources ("phytophagous strategy"), and of generalists that show variable plasticity levels in the resource they consume ("zoophytophagous strategy"). We should thus find genetic differences and a continuous variation in the diet preference within a population.

Among-individual variation in the diet may have noticeable consequences on life-history traits and thus on fitness. Compared to a diet exclusively composed of either plant or animal resources, a mixed diet generally improves adult and nymph survivorship, development time, fecundity and longevity (Naranjo and Gibson 1996; Lemos et al. 2010; Aubry et al. 2015). Furthermore, a mixed diet is required in some species to complete their development (Perdikis and Lykouressis 2004; Urbaneja et al. 2005). A poor diet deprived of one type of resource may generate a trade-off between traits (Stearns 1989; Legaspi et al. 1996). For instance, when exclusively fed on tomato fruits (*Solanum lycopersicum*), *Dicyphus tamaninii* (Wagner) (Heteroptera: Miridae) nymphs decreased their mortality rate and extended their development time compared to nymphs that consumed pure animal-based diet (i.e. whiteflies, *Ephestia* eggs, aphids and *Macrolophus caliginosus* (Wagner) (Heteroptera: Miridae) nymphs) (Lucas and Alomar 2001). Furthermore, nutrients can have different effects on different life-history traits (Catoni et al. 2008). An unbalanced diet caused by temporal and spatial changes in resource and nutrient availability, coupled with individual variation in food preferences, may result in life-history differences and trade-offs among individuals (Catoni et al. 2008).

The evolution life-history/foraging strategies requires that among-individual variations in the diet are genetically determined (Roff 2002). Genetic differences in foraging behaviour have been shown to generate variation in diet in a wide range of arthropod species (Jaenike and Holt 1991). This variation may potentially be related to life-history variation. For instance, two strains of *Drosophila tripunctata* (Loew) (Diptera: Drosophilidae) consistently exhibited preferences for different types of food resources (Jaenike 1985). Similarly, a strong genetic basis for both prey finding and consumption, and a quick response of these traits to artificial selection has been found for the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae) (Nachappa et al. 2010). In this study, selection on consumption rate induced a change in development time and fecundity, low-consumption strains having a longer development time and a lower fecundity than high-consumption strains (Nachappa et al. 2010). In contrast, evidence for genetic variation in foraging behaviour and their effects on life-history traits has yet to be found in zoophytophagous predators.

In the present study, we tested whether the zoophytophagous mullein bug *Campylomma verbasci* (Meyer) (Hemiptera: Miridae) showed genetic variation in zoophagy and whether genotypes with different levels of zoophagy adjusted their nymphal development differently when they experience a pure plant-based or a mixed animal-plant diet. The mullein bug is largely distributed among North American apple and pear orchards (McMullen and Jong 1970; Thistlewood et al. 1990; Arnoldi et al. 1992). In apple orchards, it is one of the most important predators of the European red spider mite *Panonychus ulmi* (Koch), the two-spotted spider mite *Tetranychus urticae* (Koch) (Acarina: Tetranychidae) and, to a lesser extend, the apple aphids *Aphis pomi* (de Geer) (Hemiptera: Aphididae) (McMullen and Jong 1970; Thistlewood et al. 1990; Arnoldi et al. 1992). It produces two generations every summer in East Canada (Thistlewood et al. 1990; Arnoldi et al. 1992). Individuals from the spring generation emerge early in the growing season, synchronized with both flowering apple trees and the emergence of red spider mites, and thus nymphs feed mainly on pollen, red spider mites, and/or small apples (Thistlewood et al. 1990; Arnoldi et al. 1992; Kain and Agnello 2013). Most of the adults then migrate to oviposit on an herbaceous host, mainly the mullein *Verbascum thapsus* (L.) (Scrophulariaceae) and the sumac, *Rhus tiphina* (L.) (Anacardiaceae) (Thistlewood et al. 1990; Boivin and Stewart 1982). In contrast, when born in the summer, individual mullein bugs mainly rely on the leaves and pollen of these host plants (F. Dumont, personal observations). Some individuals, however, stay on apple trees

during the summer. High spider mite densities, herbaceous hosts scarcity or risk related to migration may explain why mullein bugs stay on apple trees. In summer pollen and small fruits are no longer available on apple trees, and thus summer nymphs depend only on spider mites or aphids for their growth, as they do not feed on large apples (Boivin and Stewart 1982; Aubry et al. 2015).

We estimated the genetic basis of the number of spider mites or aphids killed per individual bug per day, and the genetic correlation between these traits and nymphal development length using isogroup lines. The occurrence of a trade-off between development length on a mixed diet and on a plant diet may have affect both the efficiency of mullein bugs to control for pests and their ability to damage apples.

Materials and methods

Mullein bug and prey populations

Mullein bugs were captured in the field as eggs, nymphs, or adult stages in different regions of Québec (Canada). In summer 2011 and 2012, nymphs and adults were captured on mullein plants (*Verbascum thapsus* L.) found in Montréal (45.53°N; −73.59°W). In late November and December 2011, we collected apple (*Malus domestica* L.) tree cuttings from orchards of the Laurentians (45.51°N; −74.03°W) and from Eastern townships (45.26°N; −72.13°W), where high-densities of red spider mites have been observed previously. We stored these cuttings at a temperature of 1 °C, in a refrigerated room. In February, cuttings were inserted into Styrofoam, put in acrylic glass cages with water, and placed in a growth chamber at 25 °C, 60 % RH, and a 16:8 L:D photoperiod. Mullein bug nymphs hatched after 10–12 days and were manually collected with a paintbrush. Each nymph was then allowed to grow in a 10 cm diameter Petri dish, with cuts of mullein, potato and soybean leaves inserted in agar gelatine and ad libitum pollen, green peach aphids *M. persicae* (referred to as aphids thereafter), and two-spotted spider mites (referred to as spider mites thereafter). Captured adults were released into a cage to reproduce (see isogroup lines below).

Both aphids and mites came from laboratory breeding stocks. Aphids were reared on potato plants (*Solanum tuberosum*) (L.) and mites on soybean plants (*Glycine max*) (L.). Stocks were kept in a growth chamber at 25 °C, 60 % RH, with a photoperiod of 16:8 L:D. Aphids stocks had been maintained on potato plants for more than five years. Two-spotted mites, were obtained from Agriculture Canada (St-Jean-sur-Richelieu, Québec, Canada), and reared for one year before the beginning of the study.

Isogroup lines

Isogroup line analysis is a method to evaluate the genetic basis of a polygenic trait, and consists of creating different lines from a small number of founder individuals (traditionally one female and one male) obtained from the studied population (Moreteau et al. 1995). The principle of the isogroup method consists in capturing the genetic variance of a given trait in a population by setting up a set of genetically different lines. Genetic variance within each line is limited by strong founder effects and genetic drift, and represents a negligible proportion of the whole population genetic variance. Because the lines are reared and tested under controlled identical conditions, the phenotypic variance among lines is mainly influenced by among-line genetic differences (David et al. 2005).

In our experiment, twelve isogroup lines of the mullein bug (referred to as line thereafter) were established from either insects captured in the field in 2012 ($\sim$300 individuals) and coming from a captive stock maintained in our laboratory since 2007 (i.e. founders were captured in a commercial apple orchard at Oka, southern Québec). For each line we included two male and two female founders. The high extinction rate of line founded by one female and one male in a pre-experiment indicated that Allee effects (Stephens et al. 1999) were probably too high to permit the individuals to meet and to reproduce. Given these strong Allee effects, we assume that only one male and one female were probably at the origin of each line. Each nymph was grown in a 10 cm Petri dish until it reached adulthood. To start a line, we released two virgin females and two males in a 30 × 30 cm acrylic glass cage each containing one mullein, one soybean, and two potato plants. Pollen, aphids, and mites were also provided ad libitum. Cages were kept in a growth chamber in the same conditions as above (except a additional 30 min pilot light to simulate twilight; copulation of mullein bug mainly occurs during twilight). Soybean and potato plants were replaced

every 7–10 days, and pollen and prey were added weekly. Lines were allowed to reproduce for at least ten generations (assuming 40 days per generation, from egg to egg). Tests were run on individuals from the first to the tenth generation (G_1–G_{10}). For each line tested individuals came from various generations (from two to eight different generations). Most lines were tested from the first or the second generation, whereas one line was tested from the fifth generation.

Zoophagy tests

Zoophagy in N3–N5 nymphs and in adults (both males and females) mullein bugs was evaluated under laboratory conditions (25 °C, 60 % RH, 16:8 L:D). In the spider mite test, we used a 10 cm Petri dish that contains fresh mullein and soybean leaves in agar on which we added a cut of soybean leaf highly infected by mites. Mites were given 24 h to move away from the fresh soybean leaf. Then, the old soybean leaf was removed from the Petri dish. Only highly infested (300 spider mites and more) soybean leaves were used to evaluate zoophagy. In the aphid test, we used a 10 cm Petri dish containing a cutting of fresh and uninfected mullein and potato leaves inserted in agar gelatine on which 30 adult female aphids were cautiously transferred with a fine paintbrush. The aphid population usually grew during the 24 h period of the test.

Bugs (both nymphs and adults) were taken from their line cages and individually placed for 24 h in a Petri dish containing a cut of fresh mullein, potato and soybean leaves inserted in agar gelatine to standardize the diet prior to the test. After that period, we transferred a bug into each of the Petri dishes using fine paintbrush, and allowed it to prey for 24 h. At the end of that period, we counted the number of prey killed (either spider mites or aphids depending on the treatment). We used between 14 and 44 individuals per line, for a total of 295 tested individuals on the spider mite diet (i.e. 23 N3 nymphs, 73 N4, 98 N5, and 97 adults), and between 15 and 35 individuals per line, for a total of 303 on the aphid diet (i.e. 48 N3, 75, N4, 85 N5 and 95 adults).

Nymphal development length

We measured nymphal development length from the beginning of N3 stage to adulthood. N1 and N2 nymphs were captured from their line cage and individually placed in 10 cm Petri dish for 24 h to standardize diet prior to beginning the test (see above). Each nymph was then transferred into another dish containing a cut of fresh mullein, potato and soybean leafs inserted in agar gelatine. In the plant treatment, ad libitum quantity of pollen was added to the dish. The mixed diet treatment consisted of ad libitum pollen, mites and aphids in the dish. Petri dishes were kept at 25 °C, 70 % RH, and a photoperiod of 16:8 L:D. Every 2–3 days nymphs were transferred into new dishes to ensure leaf freshness. Spider mites and aphids were added every two days to ensure nymphs on mixed diet always had animal resources.

We checked the development stage of each nymph every 24 h until they reach adulthood or died. Nymphal development length was calculated as the number of days from the beginning of the 3rd nymphal instar stage to adulthood. Nymphal development length was measured for 220 individuals, of which 167 reached adulthood (87 on a plant and 80 on a mixed diet). Between six and 18 individuals were recorded for each line, for a minimum of three individuals per diet and per line. Tests were run from the first to the 11th generation (assuming a generation every 40 days, Aubry personal communication).

Statistical analyses

Genetic variance in zoophagy on either spider mites or aphids was estimated using generalized mixed effect models with a log link function (GLMM). Number of spider mites or aphids killed over 24 h was analysed as a function of bug development stage and sex (N3, N4, N5, adult males or females) and generation (centred on the mean) as fixed effects. We measured generation as the number of days since the foundation of the line divided by 40 (thus assuming a generation every 40 days). We included line ID as a random effect and ran four different models (1) included line ID as the only random effect (i.e. lines differ in their levels only), (2) and (3) an interaction between line ID and generation [i.e. (2) lines differ in their slopes only, and (3) lines differ in both their levels and their slopes, respectively], and (4) random slopes and correlation between levels and slopes. Using values of generation centred on the mean allowed us to estimate among-line variance that could be estimated for in the middle of the experimental period. With the isogroup approach

we expected some divergence among lines during the study period caused by genetic drift (see below). A significant interaction between generation and line ID would indicate that lines actually diverged from each other through time. We conducted model selection by retaining the model with the lowest AIC (Burnham and Anderson 2002; Bolker et al. 2009).

We used random regression models (Dingemanse and Dochtermann 2012) to estimate genetic variance in reaction norms of nymphal development length on the two different diets. Individual values of development length were subtracted by the minimum value for that trait (i.e. nine days) and then square-root transformed to normalize the data. GLMMs on nymphal development length were run using a Gaussian distribution with diet and generation as fixed effects and line ID, diet and generation as a random effect. The random-effect structure was selected comparing the AIC of 11 models (Table 1). As above, we selected the model with the lowest AIC value. Models with line ID and with the interaction between line ID and diet provides a way to test for the significance of genotype $\times$ environment interaction in zoophagy conditional to the type of prey. Significance of fixed effects were tested using a likelihood ratio test (LRT) at a threshold of <0.05. All the tests were run by using the function *lmer* from the package *lme4* (Bates et al. 2013) in R (R Core Team 2013).

Heritability estimates

The isogroup lines approach provides an estimation of the narrow-sense heritability h^2 of the measured trait (Hoffmann and Parsons 1988). Homozygosity within each line is assumed to increase with time because of genetic drift associated with small populations, thereby reducing the genetic differences among individuals within a line (Hoffmann and Parsons 1988). Lines were reared under similar laboratory conditions, and thus among-line genetic differentiation mostly account for the variation detected among lines.

Heritability was calculated from the adjusted intra-class correlation on the latent-scale (log-normal) following the equation described in Nakagawa and Schielzeth (2010). For nymphal development length as a function of diet and generation we calculated heritability as the ratio of among-line variance over the sum of among-line and residual variance following Nakagawa and Schielzeth (2010).

Table 1 Akaike information criterion (AIC) for different generalized linear mixed-model on the number of two-spotted spider mites killed in 24 h by mullein bugs (N3 to adults) (295 individuals tested from 12 isogroup lines), the number of green peach aphids killed in 24 h by mullein bugs (N3 to adults) (n = 303), and on mullein bug's nymphal development length (from N3 until adulthood) (n = 167)

Random slopes	Random intercept	Akaike information criterion (AIC)		
		Spider mites	Aphids	Nymphal level length
	Line ID	10,034.67	902.21	**301.56**
Generation	Line ID	**9295.86**	884.06	304.65
Generation		10,181.87	969.11	309.71
Generation	Line ID	9303.65	**882.86**	303.22
Diet + generation	Line ID			303.61
Diet + generation				303.61
Diet + generation	Line ID			305.61
Diet + generation + corr	Line ID			307.79
Diet + *generation* + corr	Line ID			305.79
Diet				302.69
Diet	Line ID			302.69

The fixed structure of the model included development stage and generation (centred on the mean). The selected model, based on the lowest AIC, is in bold. Random slopes in italic were correlated to the intercept, whereas the mention "corr" indicates that the random slopes diet and generation were correlated

Genetic correlation between zoophagy and nymphal development length

We estimated genetic correlation (Spearman correlation) between zoophagy of mullein bug nymphs (adults were discarded for this analysis) on spider mites and on aphids, using best linear unbiased prediction (BLUPs) from our GLMM models. We also correlated the lines random intercepts of both models on zoophagy (i.e. zoophagy on spider mites and aphids) with lines random intercept of the model on nymphal development length. BLUPs have been shown to provide highly reliable estimates of individual intercepts in a mixed model with one random effect (Martin and Pelletier 2011).

Results

Zoophagy on spider mites and aphids

On average, a mullein bug killed 24.94 ± 2.14 (mean ± SE) mites in 24 h, for a maximum of 245 mites. Fourth instar nymphs (26.84 mites per day), fifth instar nymphs (35.16 mites per day), and adult males (22.45 mites per day) killed significantly more mites than adult females (13.60 mites per day) ($\chi^2 = 69.49$, df = 4, $p < 0.0001$). Number of mites killed by third instar nymphs (10.30 mites per day) was lower than that of adult females. Generation did not influence the number of mites killed in 24 h ($\chi^2 = 0.87$, df = 1, $p = 0.35$). The best-fitted model on the number of mites killed included lines as a random effect, the interaction between line ID and generation and a positive correlation between levels and slopes (0.78) (Table 1; Fig. 1). Among-line variance corresponded to a heritability (h^2) of zoophagy on mites of 0.54, after controlling for development stage and generation. The significant interaction between generation and line ID indicated that genetic drift was responsible for within-line changes across generations, whereas the positive correlation reveals that among-line differences in zoophagy increased with time.

N3 to N5 nymphs and adults killed on average 3.15 (±0.20 SE) aphids in 24 h, for a maximum of 18 aphids. Third (1.33 aphids per day), 4th (2.81 aphids per day) and 5th (3.60 aphids per day) instar nymphs killed significantly less aphids than adult males (3.78 aphids per day) and females (4.00 aphids per day) ($\chi^2 = 69.49$, df = 4, $p < 0.0001$). The number of aphids killed in 24 h increased with generations ($\chi^2 = 4.58$, df = 1, $p = 0.03$). The best-fitted model included lines (random intercepts) and generation (random slopes), but no correlation between them (Table 1; Fig. 2). Heritability (h^2) of zoophagy on aphids was 0.16, after controlling for development stage and generation. The random slope generation indicated that genetic drift favoured divergence among line, but it was not related to the level of zoophagy of lines.

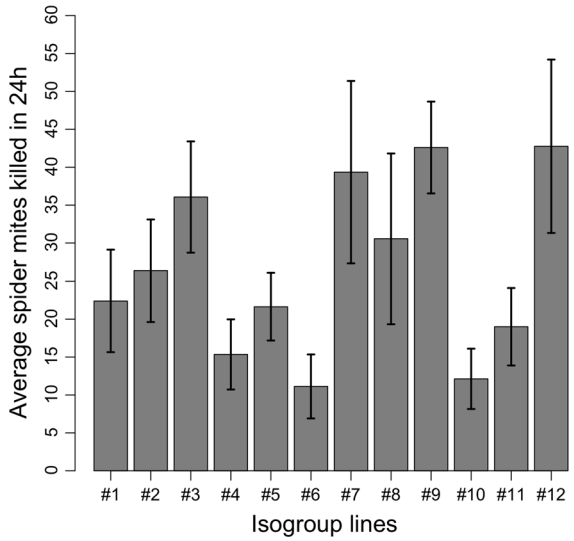

Fig. 1 Mean number of two-spotted spider mites killed per day by 12 isogroups lines of nymphs (N3–N5) and adults mullein bugs (295 individuals tested). *Error bars* correspond to SE

Nymphal development length on plant and mixed diets

Third instar nymphs reached adulthood on average 11.76 (±1.87 SD) days on a mixed diet and 12.47 (±2.13 SD) on a plant diet. Development length did not differ significantly between the diets ($\chi^2 = 2.94$, df = 1, $p = 0.09$) and among generations ($\chi^2 = 2.13$, df = 1, $p = 0.14$). The best-fitted model on nymphal development included line as random effect, but no diet or generation (Fig. 3). Lines thus differed significantly in their nymphal development length ($h^2 = 0.12$), whatever the diet.

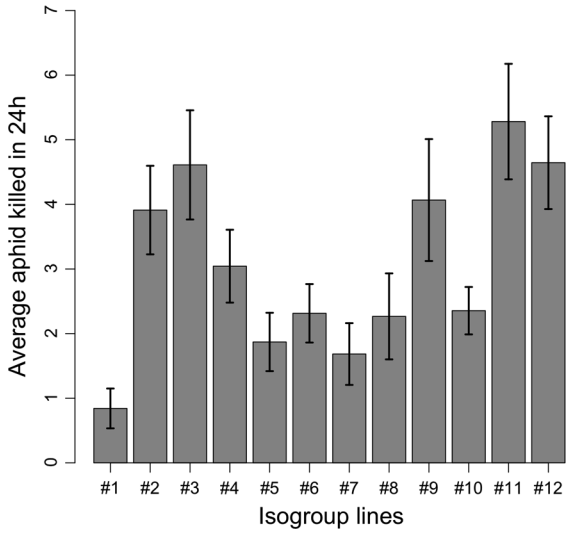

Fig. 2 Mean number of green peach aphids killed per day by nymphs (N3–N5) and adults of 12 isogroup lines mullein bugs (303 individuals tested). *Error bars* correspond to SE

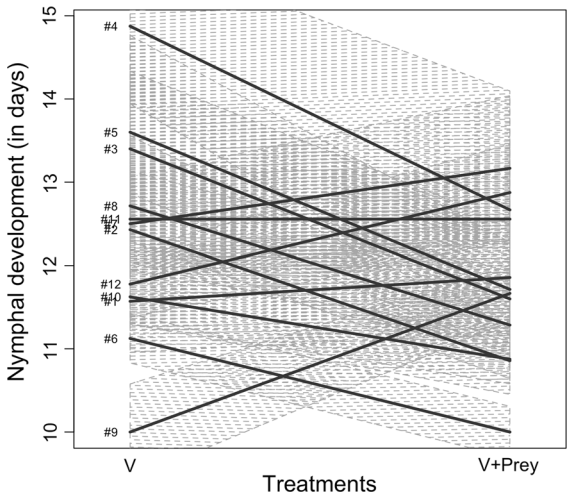

Fig. 3 Mean nymphal development length (in days) of 3rd instar nymphs of mullein bug until adulthood under a mixed (i.e. prey and pollen) diet (V + prey) or a plant diet (V) for 12 isogroup lines under laboratory conditions (25 °C, 70 % RH, 16:8 L:D). *Grey* shading represents SE for each isogroup lines. *Numbers* in the margin correspond to the isogroup line's numbers in Figs. 1 and 2

Covariance between zoophagy and nymphal development length

The level of zoophagy on mites of each line was positively correlated with zoophagy on aphids (Spearman's rho = 0.71, p = 0.01) (Fig. 4). There was no correlation between nymphal development length and either zoophagy on mites (Spearman's rho = 0.03, p = 0.92) or on aphids (Spearman's rho = 0.21, p = 0.51).

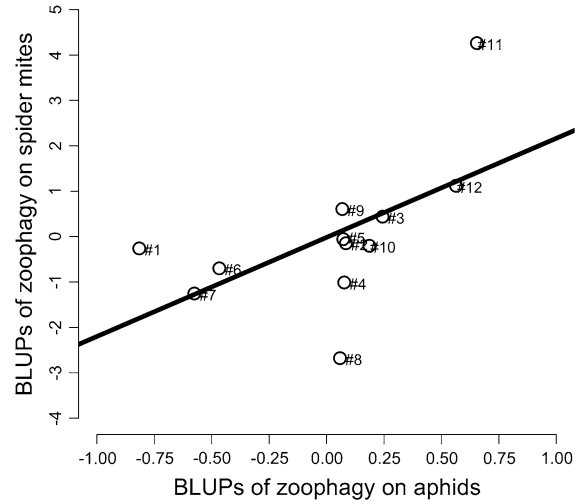

Fig. 4 Correlation between isogroup-line zoophagy on two-spotted spider mites and on aphids under laboratory conditions. Level of zoophagy was estimated as the number of prey killed per bug, per 24 h, and was controlled for nymphal stage and generation. *Numbers* beside each point correspond to the isogroup line's numbers in Figs. 1 and 2

Discussion

Foraging behaviour is expected to vary among individuals within a population, because individuals have to cope with a broad range of resources that can vary in their quality, quantity, and distribution (Bolnick et al. 2003). In the present study, zoophytophagous mullein bugs showed genetic differences in zoophagy on both mites (=primary prey) and aphids (=secondary prey). Among-line variation in the level of zoophagy may be caused by differences in their ability to detect, catch and handle a particular prey or voraciousness. The positive genetic correlation between zoophagy on both prey indicates that, overall lines differed in their level of zoophagy, and not for a specific type of prey. Differences in zoophagy could reflect differences in preference for animal versus plant resources (Svanbäck and Bolnick 2005), or various level of satiation or intake needs. We observed genetic variation in nymphal development length, but no genotype ×

environment interaction in nymphal development length. Furthermore, we did not find any evidence for a genetic correlation between zoophagy and nymphal development length. This suggests that mullein bug nymphs can fully substitute animal resources by plant material. Therefore, highly zoophagous lines cope with the absence of prey by resuming foraging on flower pollen, an abundant and easy-to-acquire source of proteins (in our experiment).

Mullein bugs have to deal with a high variability in resource availability, quality and type, both within and between generations. Spring nymphs feed mainly on pollen, red spider mites, and/or developing apple fruits (Thistlewood et al. 1990; Arnoldi et al. 1992) whereas summer nymphs have access to either pollen and mullein leaves on herbaceous hosts or prey on apple trees. Thus, genetic variance in zoophagy may reflect the coexistence of lines with different degrees of zoophagy versus phytophagy. The maintenance of such variation may be caused by the selection pressures originating from the heterogeneity of food resources in the environment. Adaptation to pollen-free environment (i.e. zoophagous strategy) may be advantageous when intraspecific competition is high (Bolnick et al. 2003), when mullein plants (or alternative herbaceous hosts) are scarce close to the orchard or when risks related to migration are high (e.g. if migration entail higher risk of predation). In contrast, the phytophagous strategy could be sustainable under high intra- versus inter-specific competition for prey resources, or under high predation risk (including the risk of intraguild predation and cannibalism). Extended period of prey scarcity (which can be caused by pest management program in agroecosystem) could also favour the phytophagous strategy and thus the proportion of phytophagous genotypes in contemporary mullein bug populations in orchards.

High level of zoophagy in some lines may also provide an adaptive advantage in environment with scarce prey resources (Maupin and Riechert 2001). For example, desert spider *Agelenopsis aperta* (Gertsch) (Arachnida: Agelenidae) populations that inhabit sites of high prey availability are less responsive to prey than populations that occupy sites with low prey availability (Maupin and Riechert 2001). In a controlled laboratory environment, Maupin and Riechert (2001) found evidence for a genetic basis to population differentiation in responsiveness to prey. In zoophytophagous predators, encounter rate with prey is obviously very low compared to encounter rate with plant resources, and less predictable (Coll 1996; Coll and Izraylevich 1997). For instance, summer mullein bug nymphs that grow on mullein plants benefit from an extended period of flowering of their hosts and proximity with the abundant plant food resources. In contrast, summer nymphs growing on apple tree have their main food resources (i.e. spider mites) distributed heterogeneously, and such a distribution may explain the high responsiveness of some genotypes as these genotypes may have an advantage in this kind of habitat. However, in our experiment we could not trace back the precise origin (i.e. mullein vs. apple tree) of the line founders, and further work is needed to test whether mullein-hosted populations are characterised by lower levels of zoophagy than populations using apple tree as a host.

To our knowledge, our results are the first evidence of genetic variation in foraging behaviour in a zoophytophagous bug. Several zoophytophagous species have important economical impact (Alomar and Wiedenmann 1996; Coll and Ruberson 1998; McGregor et al. 1999; Torres et al. 2010; Arno et al. 2010), and it could be possible to take benefit of differences in foraging behaviour within the species to improve their efficiency as biological control agent. For example, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) are released as biological control agent of white flies *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and tomato borer *Tuta absoluta* (Meynick) (Lepidoptera: Gelechiidae) in tomato field and greenhouse in Europe (Calvo et al. 2009; Luna et al. 2007; Nannini et al. 2012; Zappala et al. 2013). According to our results, the efficiency of biological control releases of *M. pygmaeus* and *N. tenuis* may be improved by artificially selecting highly zoophagous genoptypes. Consequently, fewer bug individuals would be required to achieve the expected level of pest control. For instance, Nachappa et al. (2011) observed that high voracity lines of the predatory mites *P. persimilis* at a ratio of 1:30 (predator:prey) achieved the same level of control on spider mites than unselected lines at a predator–prey ratio of 1:10. In the case of zoophytophagous predators, an increase in the ratio of predators per prey improves their efficiency as biological control agent, but it is potentially associated with increased risks of damages on the plant itself

(Sanchez 2008; Castané et al. 2011). Thus, the use of fewer genetically improved individuals would mean lower risk of damage on crop by zoophytophagous predators.

The implementation of mass-reared, genetically improved strains for either inoculative or inundative releases in biological control programs requires to take a few precautions to: (1) avoid the loss of fecundity, (2) avoid selection for detrimental genes related to the reduction of genetic variance caused by the artificial selection on the targeted trait, and (3) maintain an increased value of the target trait over several generations, under rearing conditions (Roush 1979; Hoy 1979; Hopper et al. 1993). The first two concerns can be overcame by running artificial selection on various strains, which can be occasionally mixed to increase genetic variability on untargeted traits (Nachappa et al. 2010). Moreover, the artificial condition used to achieve mass rearing should take into account the specific needs of such selected strains (ex: mobile animal prey). Finally, continuous evaluation of mass-reared populations is required to ensure quality of selected strains.

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