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Article

Impact of polymorphism and abiotic conditions on prey consumption by *Harmonia axyridis*

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With 2 figures and 2 tables

Abstract: Polymorphic diversity, such as elytra patterns of insects, is regarded as an external phenotypic characteristic driven by both genetics and environmental pleiotropy. The multicolored Asian ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae), both an efficient natural enemy of herbivores and an invasive species with a worldwide distribution, shows a multitude of elytral patterns and strong environmental adaptability. Some studies have documented differences of ecological adaptability among *H. axyridis* morphotypes, but none compared their predatory performance under differing environmental conditions, to further understand the evolutionary significance of elytral pattern diversity. We evaluated predation (number of prey consumed per time unit) on three different herbivores by melanic/succinic and male/female adults of *H. axyridis*, under different temperatures (10, 15, 20, 25, 30, 35°C). and photoperiods (4:20, 8:16, 12:12, 16:8, 20:4). We found significant differences in prey consumed by the predator between gender and polymorphic types, including under comparable environment parameters. Furthermore, there were also significant differences in predation when temperature and photoperiod regimes varied. Our results hinted a high plasticity in prey consumption relative to polymorphic type and gender in *H. axyridis*. These findings could be informative for developing further biological control programs relying on *H. axyridis*, notably for optimizing the effectiveness of predator releases according to polymorph to be used and environment targeted.

Keywords: Elytra pattern; prey preferences; prey consumption; environment conditions; phenotype fitness

1 Introduction

External polymorphic variation may be considered an evolutionary-driven phenotype since genotypic variation adjusts the fitness of animals depending on environmental conditions (Fellows et al. 2005). These external polymorphic characteristics often play an important role in assortative mating preference. This leads to regulation of population phenotype structure and directional evolution via sexual selection (Oh & Shaw 2013). The evaluation of fitness from polymorphic variation in insects under environmental conditions such as nutrition level, temperature, humidity or photoperiod, is therefore a key research area for various basic and applied issues, and further empirical experiments are required to quantify such effects (Danks 1994).

Elytra variation is one of the most visually striking of the polymorphic traits in insects, especially in certain beetles. The pattern or color of beetle elytra may vary between seasonal populations, gender and even within populations of a single niche (Crowson 1981). Documented studies of elytra pattern in Coleoptera species include the diversity of patterns (Hodek & Honek, 2009; Heslwe et al. 2010), population structure (Rodríguez-del-Bosque 2004), color biosynthesis (Arakane et al. 2010), cuticular hydrocarbons composition (Legrand et al. 2019) and pattern-related mating preferences (Jiang et al. 2007). In addition, seasonal cycles of assortative mating and reproductive behaviour may occur in polymorphic populations of predatory beetles (Wang et al. 2009). In addition, variations in prey hunting preferences among polymorphic individuals under differing environmental conditions have been investigated in many species (e.g. see Honek et al. 2017, Soares et al.2017). Such knowledge could help understanding evolutionary importance of pattern variation in elytra, including also whether nutrition is an important factor responsible (at least for some extent) in such effects.

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The Asian multi-colored ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is known for its high variation in elytra patterns as well for its voracious predatory capacity on sap-sucking insects (Lin et al. 2006, Hodek & Honek, 2009, Yu et al. 2016); it is a major natural enemy of various key pests (Obrycki & Kring 1998; Koch 2003; Ragsdale et al. 2011). In recent decades, *H. axyridis* has become also an invasive species worldwide because of its generalist feeding habits and high adaptability to various environmental conditions (Brown et al. 2008, Martins et al. 2009, Honek et al. 2016, Verheggen et al. 2017; Wang et al. 2017). Previous reports have attributed the outstanding colonization ability of *H. axyridis* to an efficient gene purifying of a highly heterozygous genome, as well as high plasticity during colonization processes (Facon et al. 2011; Tayeh et al. 2015). There has been increased attention on elytra pattern variations, notably on how they might be related to phylogeny, biology, physiology and/or ecology (Bezzerides et al. 2007, Kang et al. 2009, Orlova-Bienkowskaja et al. 2010, Guo et al. 2014). High seasonal differences in elytra patterns of *H. axyridis* populations have been reported, and it has been suggested that these occur in correspondence with adjusted structural features of populations in accordance with seasonal assortative mating preferences (Wang et al. 2009, 2011). Furthermore, there are series of specific copulatory strategies between colored males and females (Legrand et al. 2019), but at present there is little information on the predatory performance of *H. axyridis* with different elytra patterns. Increasing further our understanding of the predation behavior of *H. axyridis* showing different elytra patterns would help clarifying whether the phenotypic diversity of *H. axyridis* is beneficial not only in environmental adaptations, but also in terms of predatory activity.

Herein, we carried out laboratory experiments to compare predatory preference and prey consumption on various prey species under various season-specific environmental conditions (temperature and photoperiod), of succinic (yellow background color of elytra pattern) or melanic (black background color of elytra pattern) *H. axyridis* adults. According to previous findings on seasonal diversity of polymorphic structures within natural populations of *H. axyridis* and the assortative mating preference between two color styles, we selected temperature and photoperiod gradients to mimic with natural environmental conditions from summer to autumn in northern China. Our central hypothesis was to test whether changing temperature and/or photoperiod could influence prey choice and prey consumption of succinic and melanic *H. axyridis* (males and females).

2 Materials and methods

2.1 Insects

Two hundred pairs of *H. axyridis* of varied elytra patterns were collected from alfalfa fields in Lingshan Mountainous Natural Park, Mentougou District, Beijing, China, during May to June of 2018. The ladybirds were transported to insectaries at the Institute of Plant & Environment Protection (IPEP), Beijing Academy of Agricultural & Forestry Sciences (BAAFS) and maintained inside custom-made culturing cages (40 pairs per cage, size in 35.0 × 40.0 × 40.0 cm; aluminum column frames and 35 mesh plastic fabric nets). Artificial diet microcapsules (n = 200) (after Tan et al. 2015) were provided daily as food supply. Environmental conditions for the experimental population were controlled by an automatic indoor management system (Suntech L-100, Beijing, China) and set at: T = 25+/–1°C, RH = 65 +/–5%, illuminance = 800 lux and photoperiod of 16h L: 8h D.

We collected the aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae), adults of thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and adults of whitefly *Bemisiatabaci* Gennadius (Hemiptera: Aleyrodidae) from open field tomato plants during June 2018 in Jinfu Vegetable Farmland, Shunyi County, Beijing, China. All herbivores were transported and maintained in insectaries as described above. Approximately 80-100 herbivores of each species were placed on 6-8 young shoots of tomato with 5 main leaves each, to be used as host plants in culturing cages as above. The tomato shoots were replaced every 5 days. All experimental populations of the herbivores were reared under regulated environmental conditions using an automatic indoor management system as described above.

2.2 Predation performance under various constant temperatures/photoperiods

10-day-old adults of melanic male and female *H. axyridis* were selected from the colony and starved for 12h. Then we introduced single ladybirds into glass petri dishes (D = 15.0 cm, one ladybird per dish) with *M. persicae, F. occidentalis* and *B. tabaci* (all herbivores were 3rd instar nymphs; 100 individuals of each species) respectively. The petri dishes were placed into the artificial environment chambers (MH351, Sanyo, Japan) and temperatures set variously as 10, 15, 20, 25, 30, 35°C. Other experimental conditions were as described for population maintenance above. After 24h, the remains of herbivores in the petri dishes were counted. For both melanic/succinic and male/female adults under each temperature, we repeated above procedures for 40 replicates.

Meanwhile, we repeated the steps above except that photoperiods were set as light: dark = 4h: 20h, 8h : 16h, 12h : 12h, 16h : 8h and 20h : 4h. We also counted remains of herbivores after 24h from both ladybirds and herbivores maintained in petri dishes. For both melanic and succinic adults under each photoperiodic setup, we repeated the above procedures for 40 replicates.

3 Statistical analysis

All data obtained from experiments were evaluated by the K-S test of normal distribution. We set polymorphic type (elytra color as succinic or melanic), gender of ladybirds (male or female), prey diversity (*M. persicae, F. occidentalis* or *B. tabaci*), environmental temperature and photoperiod as independent factors in the following statistical analysis. The general linear model (GLM) was used to assess whether there were significant differences in prey hunting and consumption by different ladybird types to various herbivore species and under different environment conditions. We also tested for interactivity of these influences, between each of the two independent factors for temperature or photoperiod, respectively, by using GLM test. All statistical analyses were undertaken in R (v 3.3.2) with package glm (Tang 2008).

4 Results

4.1 Predation of polymorphic *H. axyridis* adults under various temperatures

There was a significant impact on prey consumption by the ladybird adults of different temperature regime, ladybird polymorph, gender, and prey type offered (Table 1, Fig. 1). All pairwise interactions between factors were also significant (Table 1). Succinic adults consumed more prey than melanic adults at the lowest temperatures (10 and 15°C) on the three prey types, whereas melanic adult preyed more on *M. persicae* at higher temperatures (30 and 35°C). Succinic adults also showed higher thrips consumptions at mid-level temperatures (20 and 25°C) and succinic females consumed more whiteflies than remaining polymorphic genders at both 20 and 25°C. With the temperature increasing, all 4 types of ladybirds preyed on aphids at mid-level temperatures than at low temperatures. All polymorphic male and female adult ladybirds preyed significantly less *B. tabaci* under all temperatures. Succinic male and female did not show differences in whitefly consumption, and both showed higher consumption than melanic adults.

4.2 Predation of polymorphic *H. axyridis* adults under various photoperiods

The temperature regime, ladybird polymorph, gender, and prey type offered significantly influenced the prey consumption by *H. axyridis* adults (Table 2, Fig. 2). Moreover, we also observed significant interactions between all of the pairwise independent factors (Table 2). For each prey type, all kinds of ladybirds showed the highest level of prey consumption under long light periods (L:D = 20:04), while this decreased along with shortening of light period. Further, for all photoperiod treatments and all types of ladybird adult preferred *M. persicae* than the other two prey species. Succinic ladybird adults showed higher prey consumption under mid-level (L:D = 12:12) and short (L:D = 08:16 and 04:20) light periods when they preyed on *M. persicae* and *F. occidentalis*. Moreover, we did not observe significant differences of whitefly consumption among any type of ladybird adult under shorter light period (08:16 and 04:20), whereas succinic ladybirds preyed more on *B. tabaci* under a long light period (20:04), than did melanic ones.

5 Discussion

As a foremost invasive species found worldwide, *H. axyridis* shows extremely high plasticity to various ecosystem types and environmental conditions (Moore, 1997). One mechanism conferring adaptability in *H. axyridis* might be the unusually high polymorphism of certain traits (Lombaert

Table 1. Results of the GLM used to analyze the number of prey consumed by *H. axyridis* under different temperature regimes. Main factors tested were the temperature regime ("temperature" factor: 10, 15, 20, 25, 30 and 35°C), the ladybird morph type ("polymorph" factor: succinic and melanic), ladybird gender ("gender" factor), and the type of prey offered ("prey" factor: *B. tabaci, F. occidentalis*, and *M. persicae*).

Factors	*F*	*d.f.*	*P*
Polymorph (P)	44.61	1	0.003
Gender (G)	17.54	1	0.018
Prey (PR)	31.42	2	0.006
Temperature (T)	43.14	5	0.004
P * G	22.17	1	0.010
P * PR	35.62	2	0.004
P * T	28.99	5	0.010
G * PR	22.12	2	0.011
G * T	30.62	5	0.006

Table 2. Results of the GLM used to analyze the number of prey consumed by *H. axydiris* under different photoperiods. Main factors tested were the temperature regime ("photoperiod" factor: L:D = 20:04, 16:08, 12:12, 08:16, 04:20), the ladybird polymorph type ("polymorph" factor: succinic and melanic), ladybird gender ("gender" factor), and the type of prey offered ("prey" factor: *B. tabaci, F. occidentalis*, and *M. persicae*).

Factors	*F*	*d.f.*	*P*
Polymorph (P)	33.17	1	0.005
Gender (G)	16.22	1	0.019
Prey (PR)	25.49	2	0.010
Photoperiod (PH)	43.62	4	0.004
P * G	16.74	1	0.019
P * PR	29.15	2	0.009
P * PH	29.08	4	0.009
G * PR	23.42	2	0.012
G * PH	25.18	4	0.011

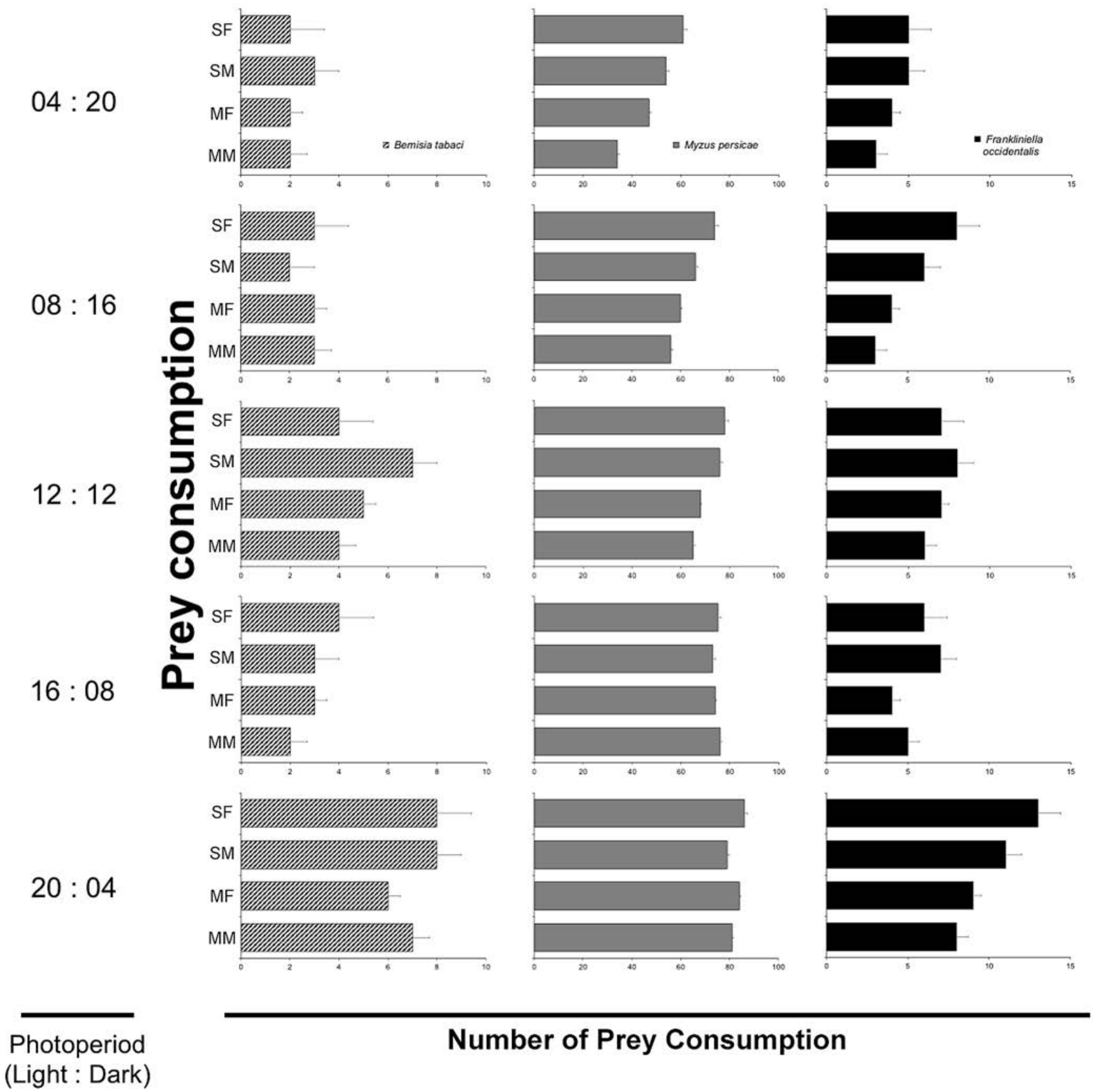

Fig. 1. Prey consumption by different *H. axyridis* morphs (SF = Succinic Female; SM = Succinic Male; MF = Melanic Female; MM = Melanic Male) to herbivores (*B. tabaci, F. occidentalis* and *M. persicae*), under different temperature regimes.

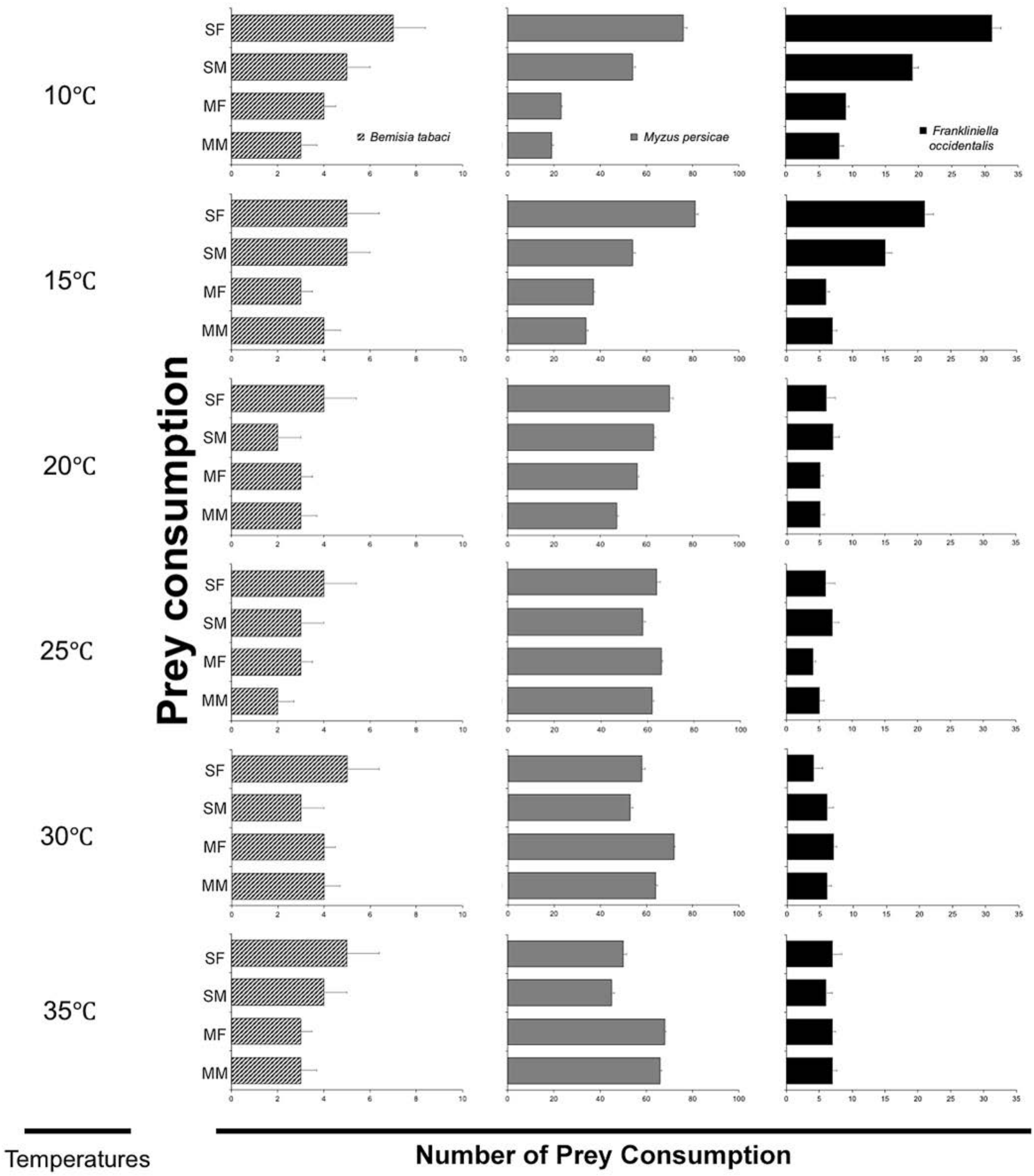

Fig. 2. Prey consumption by different *H. axyridis* morphs (SF = Succinic Female; SM = Succinic Male; MF = Melanic Female; MM = Melanic Male) to herbivores (*B. tabaci, F. occidentalis* and *M. persicae*), under different photoperiod regimes.

et al., 2008). In this study, we evaluated predation by melanic/succinic and male/female adults of *H. axyridis* on three herbivores, under various different temperature and photoperiod regimes. Our results hinted that prey consumption of ladybirds is influenced both by gender and polymorphism, even when consuming a single prey species under specific environmental conditions. Similarly, predation by different types of ladybird (succinic/melanic and male/female) to specific prey varied also under standardized temperature and photoperiod. These results indicated that polymorphic type makes some contribution to plasticity and adaptability via effects on prey consumption. These findings give some clarity in the ecological and biological mechanisms of invasiveness of *H. axyridis* in varied ecosystems.

The research presented here showed that prey consumption was temperature dependent for each herbivore tested, with preference for consumption of *M. persicae*. Succinic ladybirds consumed more prey than melanic forms at low temperatures (10 and 15°C) and melanic ladybirds showed high variability in the level of consumption of *M. persicae*, and refused to forage at lower temperatures. Many insects and other animals need an increase in food supply at certain times of year; storing energy as to maintain essential life activities during the overwintering diapause (Hahn & Denlinger 2011). Furthermore, temperature patterns have long been considered a key environmental signal which triggers biological clock processes in arthropods (Kidd et al. 2015). However, our results found variable foraging performance of *H. axyridis* polymorphs, and suggested melanic forms did not consume food in in the same way as succinic forms even under similarly cold conditions. Most studies have reported advantages of herbivore polymorphism in terms of preventing attack by predators (Bond 2006). Further, many previous reports showed diverse biological or ecological characteristics vary among *H. axyridis* phenotypes, such as prey consumption, and aspects of development and reproduction (Soares et al. 2001, 2005). Our previous survey also indicated marked differences between melanic and succinic ladybirds in natural population in patterns of mating preference (Wang et al. 2009, 2011). Mating preference of *H. axyridis* elytra patterns can be seasonally assortive (Osawa 1992, Wang et al. 2009). These polymorphic-dependent preferences indicate that the different elytra pattern types confer physiological advantages, and that population structure adjusts through sexual selection processes depending upon environmental conditions. Temperature dependent variation in hunting performance might be considered to be physiologically adaptive differences. Nutrition supply is related to many metabolic activities, such as locomotion and flight migration. Thus, further research is required to examine the mechanisms of temperature adaptive physiology and polymorphic characteristics of *H. axyridis*. Interestingly, Honek (1986) evaluated food consumption by *H. axyridis* polymorphic types via production of feces (PF). Wild collected ladybirds showed significant differences of PF between males and females, but there was little difference between ladybirds of different hostplant or color morphs. Any inconsistency in conclusions may be due to different measurements used (number of prey consumed, hunting rate or PF). Molecular metabarcoding and quantified real-time PCR methods may be applied to increase resolution on the number and types of prey consumed (Rondoni et al. 2015, Toju & Baba 2018).

Photoperiod, another environmental variable with high seasonal variability, was also shown to influence prey consumption by different polymorphs of *H. axyridis*. As with temperature, ladybirds under photoperiodic conditions simulating late autumn or winter can reduce the rate of predation. All polymorphic types of *H. axyridis* showed similar consumption rate under average (12L:12D) and long-day photoperiods (16L:8D and 12L:4D). However, with reduced light period, succinic ladybirds showed higher levels of prey consumption than melanic forms, especially females. As with temperature, a reasonable explanation for this variation in hunting by different phenotypes might be a seasonal adaptation. Components of illumination condition include light concentration, light wavelength (for colored environments) and photoperiod, all of which have been evaluated (Nation 2008). Change in photoperiod can trigger many biological or physiological adaptions in virtually all living organisms, and is oft examined in relation to seasonal signals (Ikeno et al. 2010). Thus, photoperiod is probably related to many physiological and metabolic characteristics (e.g., diapause) which provide some useful function for insects during adverse conditions such as initiation of overwintering (Hodkova & Hodek 2004; Wang et al. 2017). As mentioned above, increasing prey consumption provides energy to maintain essential life activities in cold environments. In this context, temperature is a direct factor of influence. Conversely, short-day photoperiod is not a directly negative condition for the insects but a variable that typically accompanies decreasing temperature (Smith & Smith 2012). This suggests that the varied predation strategies of ladybird polymorphic types might be an incidental effect. Similar effects on insect dispersal or migration have also be observed in response to photoperiod (Leppla et al. 1989, Nakamura et al. 1998). Such photoperiod-dependent locomotory responses might therefore always be linked with reduced food intake, and an optimal photoperiod would typically correspond to a more clement season. It is therefore essential to evaluate interactions among the genes and pathways in insects that are involved in regulation for temperature and photoperiod. This co-evolution is probably represented in many biological and physiological characteristics, not only the hunting strategy of predators. Increasing temporal and spatial scale in investigation of ecological performance of polymorphic *H. axyridis* is also necessary for enhancing our knowledge on the evolution of polymorphisms in wild populations. Furthermore, for revealing the mechanisms behind polymorphic variation and the various biological or physiological characteristics of *H. axyridis* which may be more widely relevant for understanding phenotype diversity in arthropod, experiments are required on the environmentally-driven melanic synthesis for different polymorphic types, which is viable with transcriptome and RNA interruption tests (Chen et al., 2019).

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