

Multi-generational Effects of Different Resistant Wheat Varieties on Fitness of *Sitobion avenae* (Hemiptera: Aphididae)

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Abstract

Crop resistance plays a role in preventing aphid damage, benefiting food production industries, but its effects are limited due to aphid adaptation and phenotypic plasticity. Therefore, furthering understanding of aphid–crop interactions will improve our ability to protect crops from aphids. To determine how aphids adapt to resistant varieties of wheat, *Triticum aestivum* L. over time, we performed a laboratory experiment to assess the multi-generational effects of three wheat varieties, Batis, Ww2730, and Xiaoyan22, with different resistance levels on the fitness of *Sitobion avenae* (Fab.) (Hemiptera: Aphididae). The results showed that Ww2730 and Xiaoyan22 were more resistant than Batis to *S. avenae*, regardless of whether the aphids were newly introduced or had been acclimated before being introduced to the three wheat varieties. However, the effect of resistance on aphid life-history traits was time dependent. Aphid weigh gain increased and they developed faster of the acclimated generation compared to the newly introduced generation on all three varieties. And the fecundity on the three varieties and net reproduction rates on Batis and Xiaoyan22 significantly decreased. Aphid fitness in terms of individual life-history parameters improved, whereas aphid fitness in terms of reproductive decreased, and a convergence effect, the difference gaps and standard errors of all life-history traits among the three acclimated populations had narrowed and were less than those in the three first-generation populations, was observed during the 3-mo experimental period. We suggested that *S. avenae* could rapidly respond to wheat resistance through life-history plasticity.

Key words: life-history trait, resistance, multi-generational effects, fitness

Host plants develop different resistance mechanisms to avoid phytophagous insects. In parallel, insects develop strategies to overcome these plant barriers. This is the basis of the co-evolution theory proposed by Ehrlich and Raven (1964). Aphid-resistant crop development plays a major role in preventing aphid damage and provides both ecological and economic benefits to food production, but the durability of crop resistance is limited due to aphid adaptation and phenotypic plasticity in agroecosystems (Smith and Chuang 2014, Yates and Michel 2018, Nalam et al. 2019). Therefore, furthering our understanding of aphid–plant interactions requires consideration of aphid adaptation and phenotypic plasticity.

In evolutionary theory, adaptation is a natural selection process involving biological mechanisms through which organisms

express genetic diversity and phenotypic plasticity to improve their chances of survival in a particular environment (Linhart and Grant 1996, Chevin et al. 2010). Phenotypic plasticity is the ability of a given genotype to alter its physiology, morphology, and behavior under different environmental conditions (Scheiner 1993, Via et al. 1995, Agrawal 2001, Grenier et al. 2016). Both evolutionary mechanisms are important for organisms to successfully extend their distribution into areas under different environmental conditions (Cornille et al. 2020).

The English grain aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae) is an important pest of cereals worldwide (George and Gair 1979, Gianoli 2000, Winder et al. 2012, Aradottir et al. 2017). This aphid feeds primarily on the upper leaves and ears of wheat, *Triticum aestivum* L. (Poaceae), and populations peak

following ear emergence (Watt 1979, Carter et al. 1980). Feeding aphids extract the phloem sap, thereby delaying the delivery of nitrogen components to cells, disrupting the flow of photosynthates, and limiting photosynthesis as the sooty mold spawns in the honeydew secreted on surfaces. Furthermore, the aphids transmit viruses, such as barley yellow dwarf virus, to wheat plants, which in turn reduces wheat yields (Zhou et al. 1984, Liu et al. 2014b).

Resistance in wheat plants can inhibit the growth, development, and fecundity of aphids, as well as reduce cereal aphid population increase and distribution (Watt 1979, De Zutter et al. 2012, Liu et al. 2015, Dai et al. 2016). Thus, developing resistance in wheat varieties is considered to be an efficient, economic, and environmentally friendly strategy to control cereal aphids (Dogimont et al. 2010, De Zutter et al. 2012, Crespo-Herrera et al. 2013, Shoffner and Tooker 2013, Silva et al. 2013, Dara et al. 2019). However, the aphid resistance of the main cultivated wheat varieties in China is weak, and so it is rarely relied upon to control cereal aphids (Wang et al. 2010). Contrasting results have been observed while evaluating resistance in different years, areas, and particularly between laboratory and field conditions (Watt 1979, Liu et al. 2015). Wheat varietal resistance has generally been investigated to record the individual life-history traits of aphids: seedlings were manually infested with aphids, which were allowed to feed for one generation on each wheat variety in a laboratory or greenhouse (Watt 1979, Wang et al. 2010, Silva et al. 2013, Aradottir et al. 2017). Aphids are parthenogenetic and have telescoping generations where the granddaughters of a female aphid are already developing within the daughters inside her in spring and summer (Simon et al. 2002). The alatae host preference in the English grain aphid is strongly influenced by maternal host species (Lushai et al. 1997). And the life-history traits related to individual growth and development are strongly influenced by the maternal diet, and fecundity and population parameters are strongly influenced by the offspring diet when the aphids were transferred between wheat varieties with different resistance mechanism (Hu et al. 2018). That means that the maternal effect (maternal environmental effects on offspring) of multi-generational *S. avenae* aphids needs to be controlled in host plant resistance studies as phenotypic plasticity. The natural infestation time of cereal aphids occurs over the whole living stage of wheat in the field. The field tests conducted on wheat fields involved natural or manual infestation with aphids, and results were typically recorded for 2–3 mo (Watt 1979, Liu et al. 2014b, Liu et al. 2015). This indicates that the multi-generational effect of wheat resistance to aphids was eliminated in field tests, whereas this was not the case in laboratory experiments. The multi-generational effects of insects were used to evaluate the nutritional composition of host plants and artificial diets, toxic levels of chemical substances including secondary metabolites, resistance of genetically modified crops, the nonconsumptive effects of predators on prey, and environmental pollution (Campiche et al. 2007; Smith et al. 2007; Gao et al. 2010; Kafel et al. 2012, 2014; Gao et al. 2014; Li et al. 2014; Araujo et al. 2019; Ingerslew and Finke 2020). However, these effects have rarely been used to evaluate the resistance of nongenetically modified crops to insect. In the present study, we attempted to determine the multi-generational effects of wheat varieties with different levels of resistance on aphid adaptability and phenotypic plasticity. We compared the life-history traits of *S. avenae* on three selected wheat varieties either for one generation or for acclimated generations over a period of 3 mo. The

aim of this work was to understand how aphids adapt to resistant wheat varieties over time.

Materials and Methods

Wheat and Aphids

Three winter wheat varieties, susceptible Batis and resistant Ww2730 from Germany and resistant Xiaoyan22, a high yield and adaptability cultivar widely cultivated in Shaanxi province, China, were tested. The resistance mechanism of both wheat accessions, Ww2730 and Xiaoyan22, was antibiosis. The possible resistance factor of Ww2730 was a physical factor in the epidermis and cortex, and that of Xiaoyan22 was a chemical factor in the phloem.

Here, the English grain aphid *S. avenae* was used. A single individual alate aphid was collected and cultured on wheat seedlings (*T. aestivum* ‘Costez’) in a 50 × 50 cm cage to set up a stock population. The wheat seedlings were planted in a 9 × 9 × 10 cm plastic pot filled with a mixed potting medium consisting of sand, humus, and black loam at a ratio of 1: 3: 3. The density of wheat seedlings was ~40 per pot. The pot with seedlings was changed once every 2 wk. A new pot with wheat seedlings at the two-leaf stage 13 d after sowing was moved to the cage, and ~50 nymphs (1–2 instar) were transferred from the wheat seedlings in the old pot to the wheat seedlings in the new pot. Then the old pot was moved out of the cage. The cage was placed in a 3 × 5 m plant growth chamber for >1 yr. The chamber conditions were set at 20 ± 0.5°C (day) and 18 ± 0.5°C (night), with a photoperiod of L16 h: D8 h and 70 ± 10% relative humidity. Four Sun System New Wave 48-T5 fluorescent fittings provided illumination. Each of the fluorescent fittings had eight high-output fluorescent tubes.

Experimental Design

A two-factor factorial design experiment was established. One factor was the wheat variety (among Batis, Ww2730, and Xiaoyan22), and the other factor was the feeding generation (between first and acclimated generations). There were a total of six treatments, each treatment repeated about 30 times. For each repetition, a single aphid was admitted to feed on one test seedling, and their life-history traits were monitored and recorded daily. These life-history traits among wheat varieties, generations, and treatments were compared based on ANOVA.

For the first step, ~50 nymphs at the first or second instar (G0 generation) were transferred from the stock population (Fig. 1A) to fresh wheat seedling (*T. aestivum* ‘Costez’) and allowed to grow and reproduce. Ten days later, the first generation (G1), first-instar nymphs born within 24 h were transferred to the test seedlings of the three wheat varieties at the two-leaf stage (13 d after sowing) using a small brush. These aphids were marked as B1 on Batis, W1 on Ww2730, and X1 on Xiaoyan22 (Fig. 1B). Then, their life-history traits were monitored and recorded daily. The rearing conditions and measuring methods for the first generation were the same as those for the stock population.

For the second step, ~50 first- or second-instar nymphs (G1 generation) produced by the G0 generation were transferred to fresh rearing seedlings of the three varieties to establish three independent acclimated populations in three separate 50 × 50 cm cages. Ten days later, ~50 first- or second-instar nymphs (G2 generation) produced by the G1 generation were transferred to fresh rearing seedlings for each acclimated population, and old rearing seedlings were removed from the cages. The same method was used for the next step (Fig. 1C). The cages were covered with 200-mesh gauze to ensure

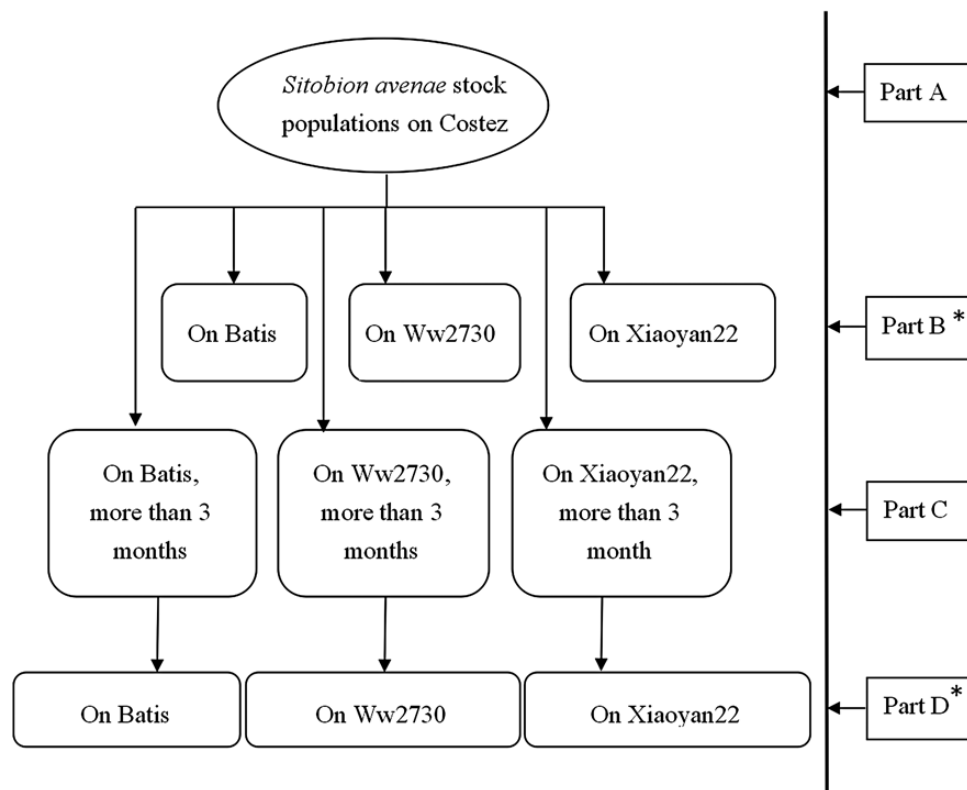


Fig. 1. Schematic overview of the experimental design. Note: Part A, the stock population (G0 generation) of the English grain aphid, *Sitobion avenae* (Fab.) was maintained on wheat, *Triticum aestivum* L. 'Costez', Part B, the life-history traits of first generations (G1) on three wheat varieties were measured, B1 is on Batis, W1 is on Ww2730, and X1 is on Xiaoyan22 individually. Part C, three acclimated populations (AG), feeding for more than 3 mo (approximately 10 generations) on three wheat varieties individually. Part D, the life-history traits of acclimated generations were measured, and marked as BB on Batis, WW on Ww2730, and XX on Xiaoyan22 individually. '*', the life-history traits measured for the aphid. The life-history traits of *Sitobion avenae* for first and acclimated generations (mean \pm SE). (A) Development time, (B) weight gain, (C) mean relative growth rate, MRGR, (D) fecundity, (E) the intrinsic rate of increase, r_m , (E) net reproduction rate, NRR. Note: G1 is first generations, AG is acclimated generations on x axis. '***' above the columns of the bar graph indicate that the difference is significant ($P < 0.01$). Two-tailed paired-sample *t*-test was used to analyze the mean differences in life-history traits between the G1 (three wheat varieties pooled together were 89 aphid samples) and AG (three wheat varieties pooled together were 90 aphid samples) after ANOVA.

that there was no mixing of the populations. Three months later, 30 adult apterae from each acclimated population were transferred to the fresh wheat seedling and allowed to reproduce. From the acclimated generation (AG), the first-instar nymphs born within 24 h, were transferred to the test seedlings, and their life-history traits were monitored and recorded daily (Fig. 1D). These aphids were marked as BB on Batis, WW on Ww2730, and XX on Xiaoyan22. The rearing conditions and measuring methods of the AG were the same as those for the stock population and first generation.

For each monitored and recorded unit, only one test seedling was planted in each plastic pot (9 \times 9 \times 10 cm), and only a single aphid was admitted to feed on one test seedling. There were 30 replicates for each treatment. To prevent aphids from escaping, the test seedling and aphid were covered with a ventilated clear glass cylinder (diameter, 4 cm, height, 24 cm). The soil surface of each pot was covered with white sand to easily detect the aphids if they fell off a plant. All aphids and test seedlings were placed in a growth chamber.

The life-history traits included (1) development time (DT) = the time from birth to adult emergence + 0.5 d (as the first-instar nymphs that transferred to the test seedlings were born within 24 h, the average age was 0.5 d), (2) weight gain (WG) = $W_a - W_n$. W_a = adult weight during the first 12 h after emergence, and W_n = first-instar nymph weight (newly born) at 24 h (an electronic balance (Sartorius MSA, Göttingen, Germany) was used to weigh the aphids), (3) fecundity = offspring produced per female within a duration that was

the same as DT after they matured, (4) mean relative growth rate (MRGR) = $(\ln W_a - \ln W_n)/DT$ (Wyatt and White 1977, Leather and Dixon 1984), (5) the intrinsic rates of natural increase (r_m) = $0.738 \ln(\text{fecundity})/(2 \times DT)$, and (6) net reproduction rate (NRR) = $\text{fecundity}/(2 \times DT)$.

Data Analyses

To determine the adaptive ways of the aphids, each of the life-history traits—DT, WG, MRGR, fecundity, r_m , and NRR—was analyzed by ANOVA using a generalized linear model with aphid generations and wheat varieties as fixed factors using SPSS 17.0 (SPSS Inc., Chicago, IL). If ANOVA showed that the difference in a life-history trait was significant, then two-tailed paired-sample *t*-test was used to analyze this life-history trait difference between the first generation and the acclimated generation in each of the three wheat varieties and in all three combined. To avoid false positives, the Bonferroni test was used to analyze the life-history trait differences among the different varieties for combined and separate generations after ANOVA. If the generation and variety had mutual effects ($P < 0.05$) in ANOVA, then the post hoc Tukey's test was used to analyze the differences in that life-history trait for all treatments.

Then, we conducted a principal component analysis (PCA) using to identify changes in aphid fitness based on individual parameters and their means on different wheat varieties over 3 mo. PCA could extract the principal components from several inter-correlated

quantitative dependent variables to display patterns of similarity in the observations and variables as points on maps. Six vectors of the life-history trait variable, DT, WG, MRGR, fecundity, r_m , and NRR, for each individual aphid and the mean for each treatment were analyzed by PCA. All statistical analyses were performed in SPSS 20.0 (Yuan and Zhou 2002, Hu et al. 2013).

Results

Life-history Trait Differences Between Combined Generations and Varieties

All life-history traits are listed in Table 1.

The DT, WG, MRGR, fecundity, and NRR (not r_m) of *S. avenae* were significantly different between the first and acclimated generations. When data for all three wheat varieties were pooled together, and the differences between the first and acclimated generations were examined, it was found that the DT was shortened by 7.48% ($F = 34.78$, $P < 0.001$, $df = 1$, 173), and WG and MRGR were increased by 20.40% ($F = 29.83$, $P < 0.001$, $df = 1$, 173) and 14.02% ($F = 54.92$, $P < 0.001$, $df = 1$, 173), respectively, in 3 mo (Fig. 2A–C). However, the fecundity and NRR were decreased by 24.62% ($F = 90.26$, $P < 0.001$, $df = 1$, 173) and 18.91% ($F = 42.28$, $P < 0.001$, $df = 1$, 173) respectively, in 3 mo (Fig. 2D and F). The r_m was not significantly changed ($F = 0.23$, $P = 0.63$, $df = 1$, 173) in 3 mo (Fig. 2E).

All life-history traits of *S. avenae* were significantly influenced by wheat variety. When data for both generations were pooled together, and the differences among the three wheat varieties were examined, it was found that the DT on Batis was shorter than that on Ww2730, and the DT on Xiaoyan22 was intermediate, with no significant difference with the DT on Batis and Ww2730 ($F = 7.16$, $P = 0.001$, Fig. 3A). The WG and MRGR on Batis were greater than those on Ww2730 and Xiaoyan22 ($F = 5.27$ and 11.66 , $P = 0.006$ and < 0.001 , both $df = 2$, 173, Fig. 3B and C). The Fecundity, r_m , and NRR on Batis were greater than those on Ww2730 and Xiaoyan22 ($F = 42.44$, 29.86 , and 48.14 ; all three: $P < 0.001$, and $df = 2$, 173, Fig. 3D–F).

For all treatments, the DT, WG, and MRGR of *S. avenae* were not significantly influenced by the interaction between wheat variety and generation (DT: $F = 1.85$, $P = 0.16$; WG: $F = 2.63$, $P = 0.08$; MRGR: $F = 1.56$, $P = 0.213$, Fig. 4A–C), but fecundity, r_m , and NRR of *S. avenae* were (fecundity: $F = 7.20$, $P = 0.001$; r_m : $F = 3.63$, $P = 0.03$; NRR: $F = 6.69$, $P = 0.002$. All: $df = 2$, 173). The fecundity of 1G on Batis was highest; the fecundity of 1G on Ww2730 and Xiaoyan22, and that of AG on Batis were lower. And the fecundity of AG on Ww2730 and Xiaoyan22 were the lowest (Fig. 4D). The r_m of 1G on Batis was the highest, but it was not significantly different from the r_m of AG on Batis. The r_m of 1G on Ww2730 was the lowest, but it was not significantly different from that of AG on Ww2730 and Xiaoyan22. The r_m of 1G on Xiaoyan22 was intermediate (Fig. 4E). The NRR of 1G on Batis was the highest, and was significantly higher than those of others. The NRR of AG on Ww2730 and Xiaoyan22 was the lowest, and it was not significantly different from that of 1G on Ww2730 (Fig. 4F).

Life-history Trait Differences Among Wheat Varieties for Both Generations and Between Generations for Each of the Three Wheat Varieties

For the first newly introduced generation when the newly introduced, all life-history traits were significantly different among the three wheat varieties (DT: $F = 6.17$, $P = 0.003$; WG: $F = 6.64$,

Table 1. The life-history traits of the first generation and acclimated generation *Sitobion avenae* on the three wheat varieties (means \pm SE)

Wheat varieties	Feeding Generation	Development time (d)	Weight gain (μ g)	Mean relative growth rate, MRGR	Fecundity	Intrinsic rates of natural increase, r_m	Net reproduction rate, NRR
Batis	First generation(30)	10.20 \pm 0.17 B	581.17 \pm 19.15 A	0.2653 \pm 0.0051 A	34.87 \pm 1.06 A	0.2576 \pm 0.0042 A	1.7144 \pm 0.0514 A
	Acclimated generation(30)	9.55 \pm 0.15	656.30 \pm 20.73	0.2901 \pm 0.0059	24.30 \pm 0.51 B	0.2480 \pm 0.0047 AB	1.2868 \pm 0.0380 B
Ww7300	First generation(29)	11.09 \pm 0.19 A	468.28 \pm 25.20 B	0.2276 \pm 0.0061 B	23.86 \pm 1.18 B	0.2109 \pm 0.0054 D	1.0911 \pm 0.0609 BC
	Acclimated generation(30)	9.92 \pm 0.16	638.83 \pm 20.40	0.2726 \pm 0.0053	20.03 \pm 0.55 C	0.2237 \pm 0.0037 CD	1.0365 \pm 0.0414 C
Xiaoyan22	First generation(30)	10.42 \pm 0.20 AB	512.00 \pm 21.61 AB	0.2446 \pm 0.0059 AB	26.30 \pm 1.03 B	0.2325 \pm 0.0055 BC	1.2793 \pm 0.0560 B
	Acclimated generation(30)	9.83 \pm 0.13	587.00 \pm 25.30	0.2788 \pm 0.0060	19.87 \pm 0.84 C	0.2237 \pm 0.0046 CD	1.0171 \pm 0.0459 C

Note: The number in the brackets behind the generation in 'Feeding Generation' column is the repeat times (aphid number) of each treatment.

This tables and Figs 2–4 represent the same data, but that for Fig. 2, three wheat varieties data of the first generation and acclimated generation were combined to focus on the difference between generations, and for Fig. 3, two generations data of the three wheat varieties were combined to focus on the difference among three wheat varieties.

The post hoc test was performed based on the results of ANOVA. DT, GW, and MRGR of the first generation were significantly different among the three wheat varieties, so the different capital letters in columns indicate significant differences of first-generation aphids among three wheat varieties based on Bonferroni test ($P < 0.01$). DT, GW, and MRGR of the acclimated generation were not significantly different among three wheat varieties ($P > 0.05$) based on Bonferroni test, so no capital letters in the columns. Fecundity, r_m , and NRR were significantly influenced by the interaction between wheat variety and generation. The different capital letters indicate significant differences among treatments based on Tukey's test ($P < 0.01$).

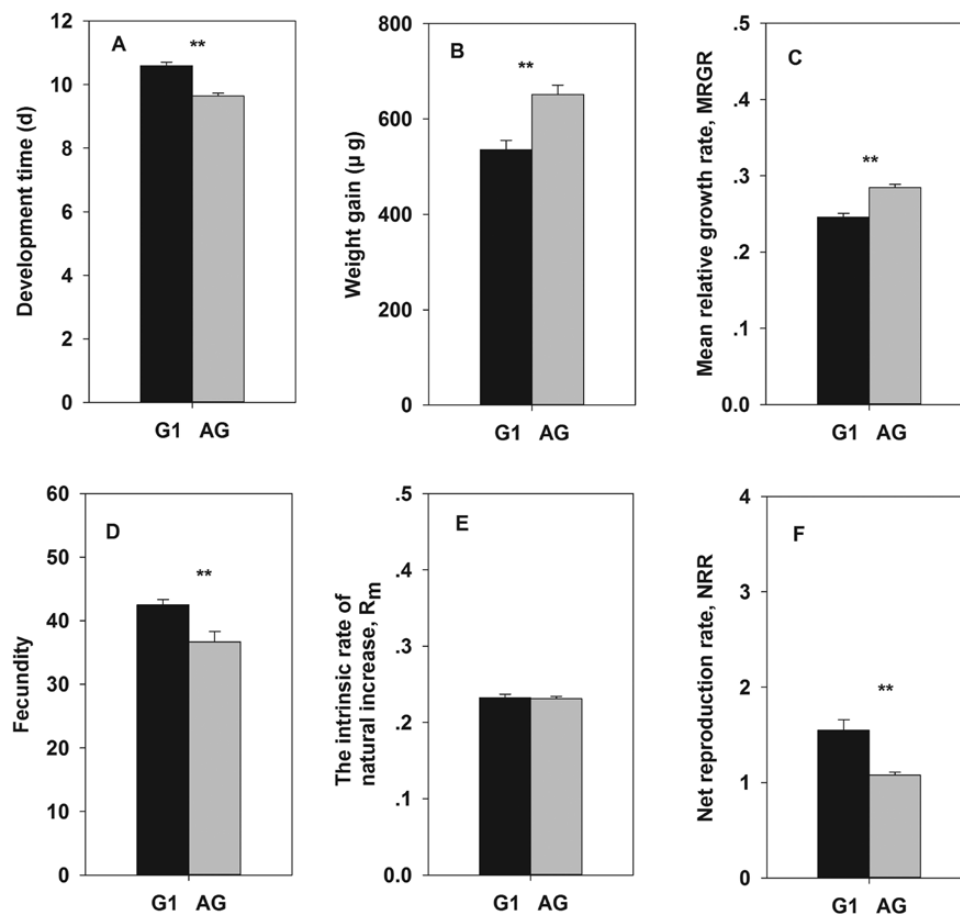


Fig. 2. The life-history traits of *Sitobion avenae* for first and acclimated generations (mean \pm SE). (A) Development time, (B) weight gain, (C) mean relative growth rate, MRGR, (D) fecundity, (E) the intrinsic rate of increase, r_m , (F) net reproduction rate, NRR. Note: G1 is first generations, AG is acclimated generations on x axis. “**” above the columns of the bar graph indicate that the difference is significant ($P < 0.01$). Two-tailed paired-sample t-test was used to analyze the mean differences in life-history traits between the G1 (three wheat varieties pooled together were 89 aphid samples) and AG (three wheat varieties pooled together were 90 aphid samples) after ANOVA.

$P = 0.002$; MRGR: $F = 10.88$, $P < 0.001$; fecundity: $F = 28.09$, $P < 0.001$; r_m : $F = 21.49$, $P < 0.001$; NRR: $F = 32.41$, $P < 0.001$. All: $df = 2, 86$). The post hoc Bonferroni test after ANOVA showed the following: The DT on Batis was significantly shorter by 8.00% compared with that on Ww2730, and the DT on Xiaoyan22 in the middle was not significantly different from that on both Batis and Ww2730 (the black columns in Fig. 4A). The WG, MRGR, fecundity, r_m , and NRR on Batis were significantly greater by 24.11%, 16.56%, 46.12%, 22.17%, and 57.13%, respectively, than those on Ww2730, and by 13.51%, 8.48%, 32.57%, 10.79%, and 34.02%, respectively, than those on Xiaoyan22 (the black columns in Fig. 4B–F).

For the acclimated generation, the DT, WG, and MRGR were not considerably different among the three wheat varieties (DT: $F = 1.74$, $P = 0.181$; WG: $F = 1.97$, $P = 0.146$; MRGR: $F = 2.37$, $P = 0.099$; All: $df = 2, 87$). The gray bars are shown in Fig. 4A–C). However, the fecundity, r_m , and NRR were considerably different among the three wheat varieties (fecundity: $F = 15.02$, $P < 0.001$; r_m : $F = 10.32$, $P < 0.001$; NRR: $F = 16.41$, $P < 0.001$; All: $df = 2, 87$). The post hoc Bonferroni test after ANOVA showed as the following: The F , r_m , and NRR on Batis were greater by 21.30%, 10.88%, and 26.72%, respectively, than those on Ww2730, and by 22.32%, 10.91%, and 26.51%, respectively, than those on Xiaoyan22 (the gray bars in Fig. 4D–F).

Comparing the life-history trait differences between generations for each of the three wheat varieties, it is found that the DT decreased from the first generation to the acclimated generation by 6.37%, 10.54%, and 5.61% on Batis ($F = 8.46$, $df = 1, 57$, $P = 0.005$), Ww2730 ($F = 22.29$, $df = 1, 58$, $P < 0.001$), and Xiaoyan22 ($F = 6.21$, $df = 1, 58$, $P = 0.016$), respectively, which indicates almost an entire day (compared adjacent black and gray bars are shown in Fig. 4A; the same below). WG significantly increased from the first generation to the acclimated generation by 12.93%, 36.42%, and 14.65% on Batis ($F = 4.51$, $df = 1, 58$, $P = 0.038$), Ww2730 ($F = 26.75$, $df = 1, 57$, $P < 0.001$), and Xiaoyan22 ($F = 5.08$, $df = 1, 58$, $P = 0.028$), respectively (Fig. 4B). MRGR increased from the first generation to the acclimated generation by 9.43%, 19.74%, and 13.88% on Batis ($F = 10.02$, $df = 1, 58$, $P < 0.001$), Ww2730 ($F = 31.22$, $df = 1, 57$, $P < 0.001$), and Xiaoyan22 ($F = 16.54$, $df = 1, 58$, $P < 0.001$), respectively (Fig. 4C). However, the fecundity of the acclimated generation was lower by 30.31%, 16.05%, and 20.48% compared with that of the first generation on Batis ($F = 81.10$, $df = 1, 58$, $P < 0.001$), Ww2730 ($F = 8.85$, $df = 1, 57$, $P = 0.004$), and Xiaoyan22 ($F = 23.32$, $df = 1, 58$, $P < 0.001$), respectively (Fig. 4D). The r_m was not significantly changed on Batis ($F = 2.31$, $df = 1, 58$, $P = 0.134$), Ww2730 ($F = 3.89$, $df = 1, 57$, $P = 0.053$), and Xiaoyan22 ($F = 1.06$, $df = 1, 58$, $P = 0.221$; Fig. 4E). The NRR of the acclimated generation was lower by 24.91% and 24.46% compared with those of the first generation on Batis ($F = 44.76$, $df = 1, 58$, $P < 0.001$)

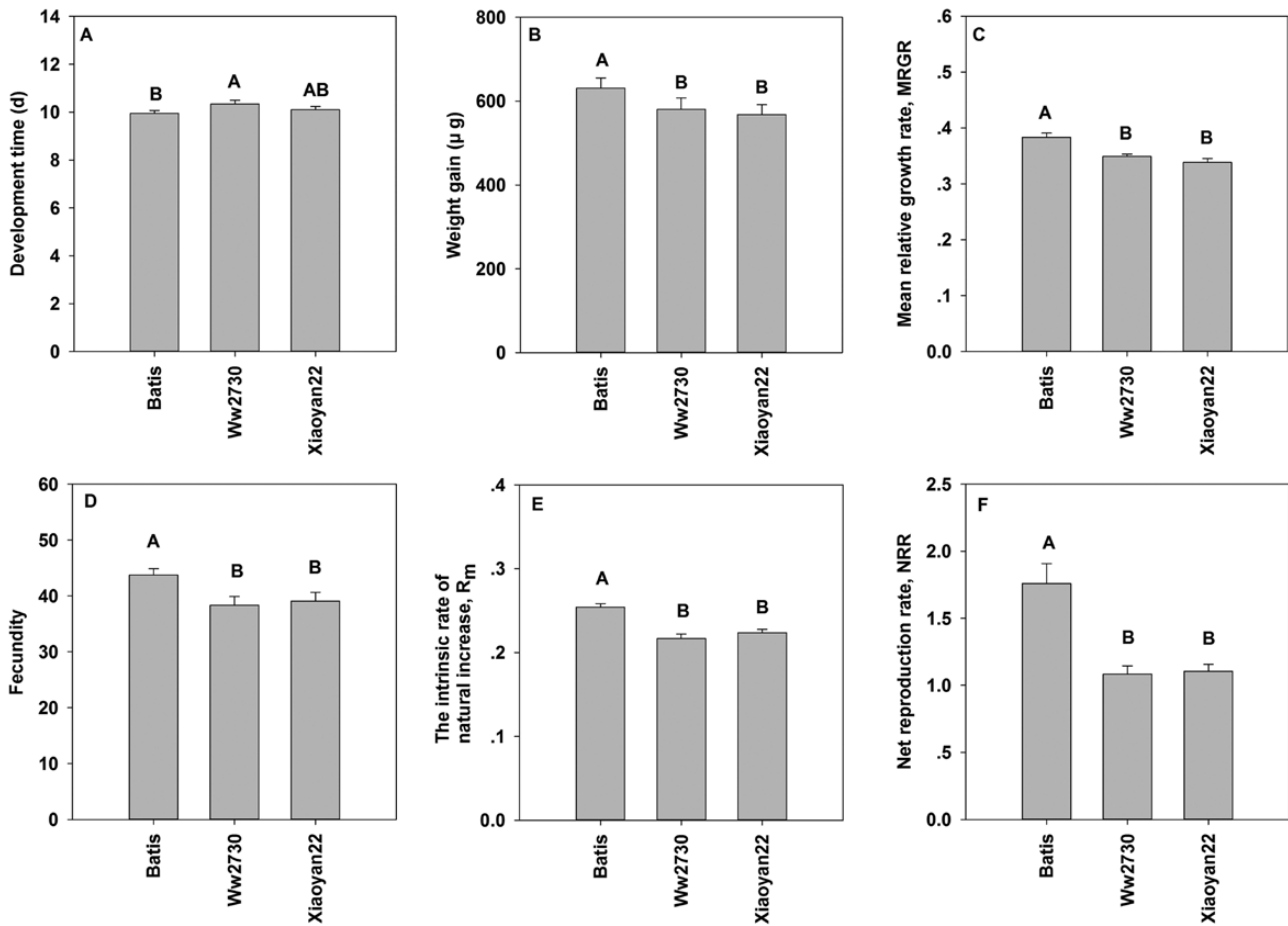


Fig. 3. The life-history traits of *Sitobion avenae* on three wheat varieties (mean \pm SE). (A) Development time, (B) weight gain, (C) mean relative growth rate, MRGR, (D) fecundity, (E) the intrinsic rate of increase, r_m , (F) net reproduction rate, NRR. Note: The same capital letters among the columns of the bar graph indicate that the difference is not significant ($P > 0.05$), and different capital letters indicate that the difference is significant ($P < 0.01$). Bonferroni test was used to analyze the differences in life-history traits among different varieties for combined generations (first generation and acclimated generations pooled together, Batis has 60 aphid samples, Ww2730 has 59 aphid samples, and Xiaoyan22 has 60 aphid samples) after ANOVA.

and Xiaoyan22 ($F = 13.11$, $df = 1, 58$, $P < 0.001$), but there was no significant difference on Ww2730 ($F = 1.27$, $df = 1, 57$, $P = 0.27$; Fig. 4F).

Fitness Change in 3 mo

PCA1 and PCA2 explained 65.08% and 26.04% of the variance, respectively, based on 179 samples of the first and acclimated generations (Fig. 5A). $PCA1 = -0.740 DT + 0.824 WG + 0.840 MRGR + 0.608 fecundity + 0.968 r_m + 0.816 NRR$. $PCA2 = 0.569 DT - 0.236 WG - 0.480 MRGR + 0.789 fecundity + 0.075 r_m + 0.571 NRR$.

PCA1 and PCA2 explained 55.55% and 42.74% of the variance, respectively, based on the average vectors of three first generations and three acclimated generations (Fig. 5B). $PCA1 = -0.901 DT + 0.893 WG + 0.912 MRGR + 0.227 fecundity + 0.811 r_m + 0.429 NRR$, which mainly represented the growth and development of *S. avenae* and increased on all three wheat varieties over 3 mo. $PCA2 = 0.411 DT - 0.390 WG - 0.390 MRGR + 0.972 fecundity + 0.576 r_m + 0.903 NRR$, which mainly represented the population parameters and decreased markedly on all three wheat varieties over 3 mo.

Discussion

English grain aphids are highly adaptable to different hosts and exhibit high levels of phenotypic plasticity to adapt to novel hosts or host resistance (Dai et al. 2016; Hu et al 2018). In the present study,

when the aphids were allowed to feed for one generation on wheat seedlings (first generation), Ww2730 and Xiaoyan22 exhibited significantly higher levels of resistance against *S. avenae* than did Batis. Low values for fecundity, WG, MRGR, r_m , and NRR, and longer DT indicated that aphid fitness was lower on the wheat varieties. Of the three wheat varieties tested, the shortest DT and largest WG, MRGR, fecundity, r_m , and NRR were observed on Batis. Three months later, the fecundity, r_m , and NRR of the acclimated generation on Batis were still greater than those on Ww2730 and Xiaoyan22. However, the DT, WG, and MRGR of aphids in the acclimated generation were similar, and the differences gap in fecundity, r_m , and NRR among the three acclimated generation populations narrowed. The standard errors of all six parameters in the acclimated generation were also lower than those in the first generation. When comprehensively evaluated by PCA, a convergence effect was observed for *S. avenae* to adapt to the wheat varieties with different levels of resistance during the 3 mo. It was reported that plant historical domestication, foreign crops, and resistant cultivar introduction all led to changes in the quality, defenses, and availability of host plants (Peccoud et al. 2010, Züst and Agrawal 2016, Simon and Peccoud 2018). These changes imposed considerable selective pressure on aphid populations, and aphid populations rapidly responded to these selective pressures, such as insecticide resistance, new plants, and natural enemies in the agroecosystems (Peccoud et al. 2010, Züst and Agrawal

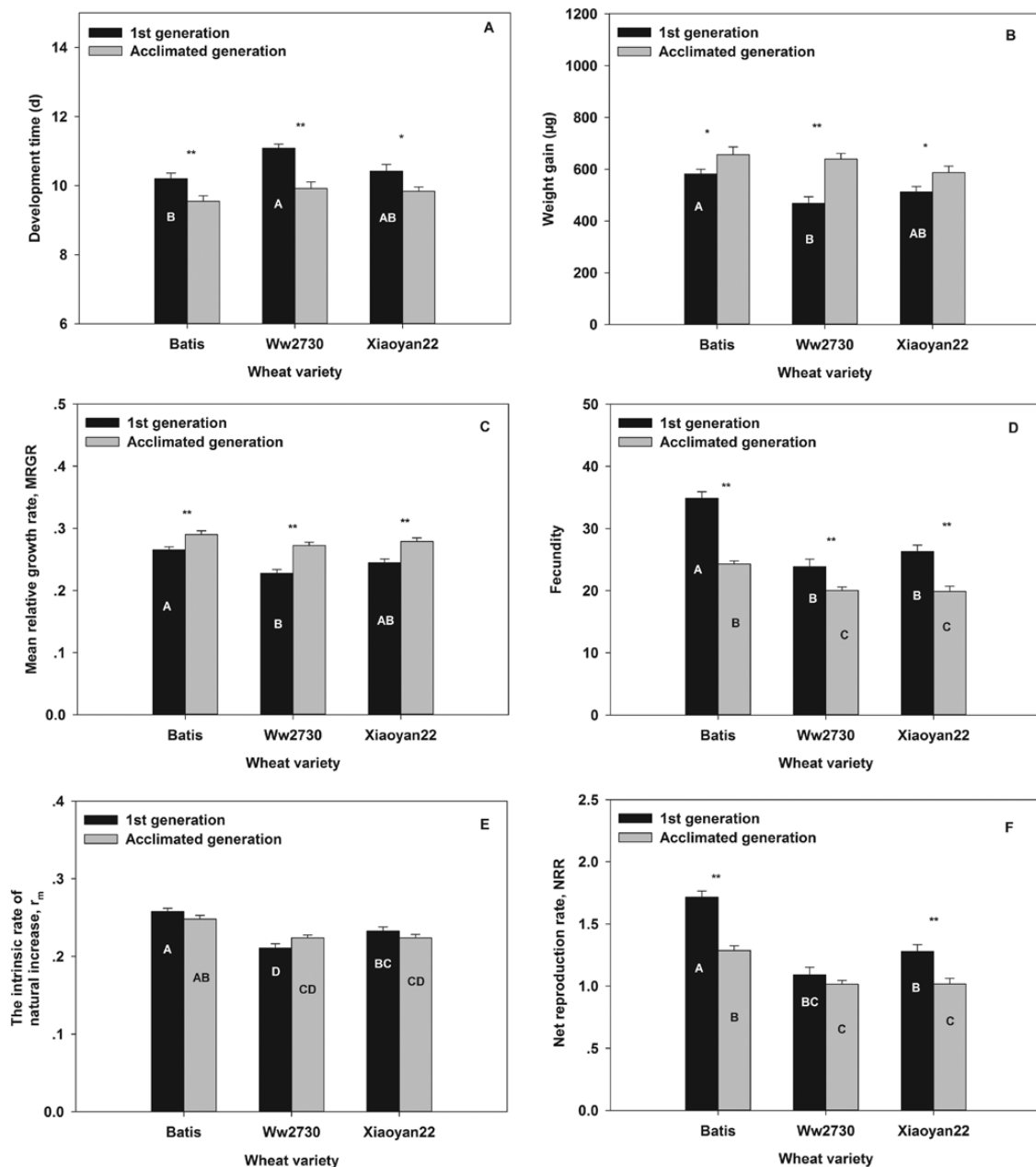


Fig. 4. The difference in life-history traits for *S. avenae* between first and acclimated generations on three wheat varieties (mean \pm SE). (A) Development time, (B) weight gain, (C) mean relative growth rate, MRGR, (D) fecundity, (E) the intrinsic rate of increase, r_m , (F) net reproduction rate, NRR. Note: The repeat times (aphid number) of each treatment were 30, beside first generation on Ww2730 was 29. The same capital letters in the columns of the same color bar graph indicate that the difference is not significant ($P > 0.05$), and different capital letters indicate that the differences between parameters of the first-generation aphid, or acclimated generation, or each treatment are significant among the three wheat varieties ($P < 0.05$). '**' indicates significant difference ($P < 0.05$) between 1G and AG, '***' indicates significant difference at $P < 0.01$ between 1G and AG. Fecundity, r_m , and NRR were significantly influenced by the interaction between wheat variety and generation, so the statistical results shown in the D–F show the differences among different treatments based on Tukey's test. DT, GW, and MRGR of the first generation were significantly different among the three wheat varieties, so the letters in the black columns of A–C show the differences in the first generation among the three wheat varieties based on Bonferroni test. DT, GW, and MRGR of the acclimated generation were not significantly different among the three wheat varieties (gray columns in A–C).

2016, Simon and Peccoud 2018). We found that *S. avenae* could rapidly respond to the resistance of some wheat varieties in 3 mo in the laboratory. The question then was whether this response was due to selective pressure or phenotypic plasticity. The aphids tested in the present study were derived from one single individual, hence all the populations were homogeneous. This may indicate that the adaptive response was due to phenotypic plasticity in the acclimated populations.

The physiology, morphology, and behavior of organisms can alter due to environmental conditions over short and long time scales, and even over generations (Schlichting 1986, Agrawal 2001, Srinivasan and Brisson 2012). Compared with the first generation, the life-history traits related with individual growth and development improved remarkably. The DT decreased, and WG and MRGR increased on the three wheat varieties in 3 mo. These results indicated that the aphid nymphs developed more rapidly and gained

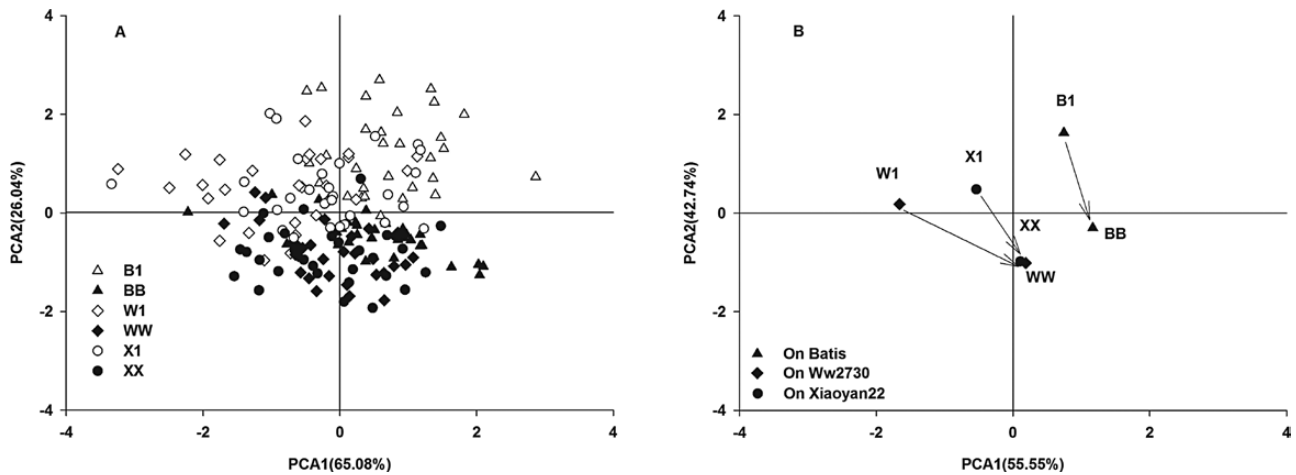


Fig. 5. The fitness change for *Sitobion avenae* feeding on three wheat varieties over 3 mo by PCA. (A) based on data of 179 individual aphids, (B) based on summary data from 6 treatments. Note: B1, first-generation aphids on Batis, BB, acclimated generation aphids on Batis; W1, first generation aphids on Ww2730, WW, acclimated generation aphids on Ww2730; X1, first generation aphids on Xiaoyan22, XX, acclimated generation aphids on Xiaoyan22.

more weight in the 3-mo period. Our results are consistent with those for *Helicoverpa armigera* (Hübner) that were reared on transgenic insect-resistant cotton, *Gossypium hirsutum* L. for over 16 generations, which showed reduced larval mortality and increased body size and body weight than those for only 1 generation (Shu et al. 2001). Our results are also consistent with those reported for later *Rhopalosiphum padi* (L.) generations, which had higher MRGR compared with earlier generations (the offspring of emigrants, alate individuals from bird cherry, *Prunus padus* L.) on both seedling and flowering stages of oat, *Avena sativa* L. (Leather 1982).

However, there was significant reduction in the traits related to population and fecundity in the acclimated generation. Interestingly, the reductions were caused by the fecundity and NRR of the acclimated generation. This result contradicted previously reported data; for example, the reproductive rates were not different between later *R. padi* generations and earlier generations (the offspring of alate individuals from *P. padus*) on both seedling and flowering stages of *A. sativa* (Leather 1982). Furthermore, the effect of Bt rice on the non-target pest brown planthopper (BPH), *Nilaparvata lugens* (Stål), over four generations and on *Anagrus nilaparvatae* Pang et Wang with parasitizing eggs of *N. lugens* over 11 generations was negligible (Gao et al. 2010, Chen et al. 2011, Liu et al. 2014a). The reproduction of the soybean cyst nematode *Heterodera glycines* (HETDGL) did not increase on six dry bean varieties during two 11-mo periods (Pormarto et al. 2011). However, our results are consistent with those for the BPH, *N. lugens*, that were reared on rice variety Minghui 63 with a introgressed resistance gene *Bph15* for over seven generations, which showed significant decrease of the life parameters relative with fecundity and ovary development (Li et al. 2014).

Our results showed that *S. avenae* responded to wheat resistance through the plasticity of their life-history traits. These traits not only included those related to individual growth and development but also those related to population development (fecundity, r_m , NRR). However, the increase in individual traits and decrease in population traits were found on both resistant and susceptible wheat varieties. The possible resistance mechanism of Ww2730 to *S. avenae* involved physical feeding restriction factors in the epidermis, mesophyll, and phloem; and that of Xiaoyan 22 involved a chemical factor in the phloem. The differences in resistance mechanisms may

be the main factor affecting the plasticity of different phenotypes. However, the effect of the resistant characteristics of wheat varieties (lines) on the life traits of aphids over time needs further research.

The effect of wheat resistance on aphids always differed between laboratory and field experiments (Liu et al. 2015). There were many differences between laboratory and field experiments when we evaluated the resistance of wheat varieties to *S. avenae*. The temperature and light are always constant in the laboratory but not in the field. The aphids always manually infested wheat seedlings in the laboratory, and aphid generations were tracked over the entire growth stage of wheat in field studies. We know that the plant defense responses induced by aphid infestation and defensive secondary metabolite synthesis and accumulation differ during different wheat growth stages. This is an important reason for the conflicting results obtained between laboratory and field studies. However, in the present study, we found that almost all life-history traits were different between the first and acclimated generations, and there was a convergence effect on all three wheat varieties with different levels of resistance in 3 mo. This result provides evidence that the multi-generational effects reflect another important reason for the conflicting results between laboratory and field studies of the resistance of wheat varieties to *S. avenae*.

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Author Contributions

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