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Assessment of fitness costs of resistance against the parasitoid *Leptopilina victoriae* in *Drosophila bipectinata*

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Abstract How insects evolve resistance or counter-resistance against antagonists is a basic issue in the study of host-parasitoid coevolution. One of the factors that affect their coevolution is fitness costs of resistance and counter-resistance. Here, we assess fitness costs of resistance against the parasitoid *Leptopilina victoriae* in *Drosophila bipectinata* on the basis of selection experiments. We made a base population by mixing three geographic fly populations that differed in resistance. After six generations of free mating, the base population was divided into four populations, two for selection of resistance against a *L. victoriae* population and two for control. Resistance increased rapidly in response to selection and reached a very high level within four generations in the two replicated selected populations, while resistance of the control populations remained low at least for 20 generations. High resistance of the selected populations was maintained at least for 10 generations even if selection was stopped. Comparison of life history and stress tolerance revealed that both selected populations had lower female longevity than the two control populations, and at least one of the selected populations had shorter thorax length and lower female desiccation tolerance and adult heat tolerance than both or either of the control populations. On the other hand, selected populations had higher male starvation tolerance and longevity than control populations. There were no significant differences in resistance against another population of *L. victoriae* and two other parasitoid species between the selected and control populations. These results suggest that the resistance against the *L. victoriae* population in *D. bipectinata* may incur some but not so high costs and act parasitoid-species- and/or parasitoid-population-specifically.

Key words Artificial selection • coevolution • specificity • trade-offs

Introduction

All insects have immune systems to defend themselves from infection of pathogens or parasites. However, their immune systems are not always effective, because some pathogens and parasites have means to avoid being detected by the host immune systems or suppress host immune responses (Edison et al. 1981; Shelby and Webb 1999; Eleftherianos et al. 2007). To cope with such enemy's adaptations, host insects often intensify their immune responses or modify their immune systems (Strand and Pech 1995; Carton et al. 2008). One of the important factors that affect such parasitoid-host coevolution is the costs of resistance and counter-resistance (Doebeli 1997; Sasaki and Godfray 1999). A powerful tool to examine these costs is the study of correlated responses to artificial selection. Kraaijeveld and Godfray (1997) and Fellowes et al. (1998) selected *Drosophila melanogaster* Meigen for improved resistance against *Asobara tabida* (Nees von Esenbeck) and *Leptopilina boulardi* (Barbotin, Carton and Kelner-Pillault), and found that the selected populations were inferior in competitive ability (survival under severe intraspecific competition) than the control populations. Fellowes et al. (1999) further indicated that lower competitive ability of the selected populations was associated with reduced rates of larval feeding. In addition, Kraaijeveld et al. (2001) found that the selected populations have approximately twice the density of haemocytes than the control populations. On the other hand, males of the selected populations achieve a higher mating success than those of control populations (Rolff and Kraaijeveld 2003), suggesting an improvement of at least one aspect of fitness in

the selected populations.

It has also been revealed in the study of Kraaijeveld and Godfray (1999) that the populations selected for resistance against an *A. tabida* population are also resistant to conspecific parasitoid populations from different geographic regions. However, fly populations resistant to a parasitoid population are not always resistant to other conspecific parasitoid populations. For example, some geographic populations of *D. melanogaster* from Africa show different responses to different geographic populations of *L. boulardi* (Dubuffet et al. 2007). In addition, the populations selected for resistance against *A. tabida* show no increase in resistance to *L. boulardi*, although the populations selected for resistance against *L. boulardi* show some increase in resistance against *A. tabida* compared with the control populations (Fellowes et al. 1999). Thus, the resistance mechanism of *D. melanogaster* has parasitoid-species- or parasitoid-population-specific components.

The above selection studies were based on within-population genetic variation. Resistance and counter-resistance against antagonists often show more extensive variation geographically (Carton et al. 1992; Kraaijeveld and van Alphen 1994; Dupas et al. 1998; Hufbauer 2001). For example, an African population of *D. melanogaster* has complete resistance against a population of the parasitoid *L. boulardi*, whereas another population has no resistance against the same parasitoid population (Dupas et al. 1998). Such geographic variation may not be a simple extension of within-population variation, but may differ in the kind or function of responsible genes. However, there has been no selection study based on geographic variation.

In this study, we assess fitness costs or trade-offs associated with resistance of *Drosophila bipectinata* Duda against the parasitoid *Leptopilina victoriae* Nordlander using host populations from different geographic regions. *D. bipectinata* occurs throughout Southeast Asia, extending into South Pacific islands, Australia, India and Africa (Bock and Wheeler 1972; Lemeunier et al. 1986; Kopp and Barmia 2005). Novković et al. (2012) reported that a *D. bipectinata* population from Iriomote-jima (southernmost Japan) is susceptible to *L. victoriae* from Kota Kinabalu (Malaysia), but its populations from Kota Kinabalu and Bogor (Indonesia) are resistant to this parasitoid population. Our preliminary study suggests that resistant populations of *D. bipectinata* show no melanization against *L. victoriae* eggs or embryos (Takigahira, unpublished data), suggesting that this species has a different resistance mechanism from *D. melanogaster* that shows a melanization response to parasitoids.

We produced a base population of *D. bipectinata* by mixing geographic populations from Iriomote-jima, Kota Kinabalu and Bogor, and selected for resistance against *L. victoriae* from Kota Kinabalu. To assess fitness costs that are associated with the resistance, we compared life history and stress tolerance traits widely between the selected and control populations, because it cannot be predicted what traits will exhibit trade-offs with parasitoid resistance. We also compared resistance/susceptibility against another population of *L. victoriae* and two other parasitoid species whether the resistance is under trade-off with resistance against other parasitoid species or population.

Materials and methods

Selection for parasitoid resistance

The base population for the selection experiments was produced by mixing three populations of *Drosophila bipectinata* derived from females collected from Bogor (BG, Indonesia: 6.6 °S, 106.8 °E) in June 2008, Kota Kinabalu (KK, Malaysia: 5.3 °N, 117.4 °E) in March 2008, and Iriomote-jima (IR, Japan: 24.4 °N, 123.9 °E) in March 2005. The *D. bipectinata* populations from BG and KK (*D. bipectinata* BG and KK) are resistant to *L. victoriae* KK, while *D. bipectinata* IR is susceptible (Novković et al. 2012). These populations were maintained on Drosophila medium under 15L: 9D (15 h light: 9 h dark) at 23°C in laboratory for a few years. To establish the base population, 20 females and 20 males from each stock population were mixed and maintained with free mating for six generations before starting the selection experiments.

The base population of *D. bipectinata* was divided into four experimental populations, two for selection of resistance against *L. victoriae* KK and two for control. The *L. victoriae* KK population used for the selection experiments originated from females collected in Kota Kinabalu in March 2008 and maintained in mass culture (100 – 200 females in each generation) using *Drosophila simulans* Sturtevant (originated from Sapporo, Japan) as host.

Selection was performed as follows. One- to two-day old *D. bipectinata* larvae were placed in a Petri dish containing a small amount of rearing medium and

then exposed to several female wasps, whose oviposition behavior was followed under a stereoscopic microscope. Characteristic oviposition behavior, such as full extension of the ovipositor after contact with host and longer insertions of the ovipositor into larvae (>10 s) were taken as indicators of successful oviposition. When oviposition was confirmed, parasitized fly larvae were transferred into vials containing Drosophila medium. Thus, all larvae were subjected to single parasitism (parasitized by one parasitoid individual). One hundred parasitized larvae were prepared for each selected population and survivors were collected to produce the next generation. The number of survivors (i.e., the number of individuals to produce the next generation) was 20-25 in the first few generations of selection but soon increased over 40. Thus, , The selected populations were maintained without selection after 10 generations of selection.

The control populations were maintained without parasitism treatment; i.e. 100 larvae were randomly chosen for each control population and flies that emerged were collected to produce the next generation. The control populations were also monitored for the resistance against *L. victoriae* KK every generation in the first 10 generations and at the 20th generation; 100 parasitized larvae were prepared for each control population and the number of flies and wasps that emerged were counted.

Female wasps used for selection and monitoring the control populations were always taken from the stock population of *L. victoriae* KK maintained using *D. simulans* as host; i.e., they had not experienced coevolutionary interactions with *D. bipectinata* at least in the laboratory. Usually more than 10 female wasps were used to prepare 100 parasitized *D. bipectinata* larvae to avoid a bias due to the individual

variation of virulence in wasps. The resistance was determined by the following equation; resistance index = $F/(F+W)$, where F was the number of flies that emerged and W was the number of wasps that emerged. The experimental populations were also maintained under 15L: 9D at 23°C.

Measurements of life history and stress tolerance traits

To assess the cost associated with parasitoid resistance, unparasitized individuals of the selected and control populations were measured for the following life history and stress tolerance traits after eight or nine generations of experimental treatments (with/without selection). In addition, life history and stress tolerance traits of the three original populations were measured for reference. Except individuals examined for the competitive ability, those used for the measurements of life history and stress tolerance traits were reared at a low density (<50 larvae per 10 ml Drosophila medium) to minimize harmful effects of high density.

Egg-to-adult development time and viability. Adult flies were introduced into vials (50 ml) with Drosophila medium and allowed to oviposit for 6 hours. Eggs were collected, introduced into new vials (25 eggs per vial) with Drosophila medium (10 ml), and placed under a continuous light at 23°C. Flies that emerged from vials were counted every 6 hours. Five replicates were prepared for each population.

Longevity. Newly eclosed flies were transferred into new vials with Drosophila medium under a continuous light at 23°C. Flies were transferred into new

vials every one or two day(s) and survivors were counted every day. Measurements were made with three replicates, each with 15~20 individuals of each sex from each population.

Female fecundity. One newly eclosed virgin female and two males were randomly paired and introduced into a vial (30 ml) with Drosophila medium (6 ml) under a continuous light at 23°C. Flies were transferred into a new vial every day, and eggs oviposited in the old vial were counted. Measurements were made for 20 days with 21 replicates for each population.

Thorax length. Adult flies were collected from vials used in the above "development time and viability" experiments, placed in vials with Drosophila medium for 2 or 3 days, and fixed in 70% ethanol. Thorax length was measured for approximately 30 individuals of each sex from each population.

Larval competitive ability. In their study using *D. melanogaster*, Kraaijeveld and Godfray (1997) assessed intraspecific competitive ability; i.e., competitive ability against a conspecific mutant strain. In the present study, however, competitive ability against a different species, *D. simulans*, was assessed, because an appropriate mutant strain was not available in *D. bipectinata*. Both of *D. simulans* and *D. bipectinata* are fruit-feeders mainly exploiting succulent fruits (Hirai et al. 2000; Mitsui and Kimura 2010; Novković et al. 2012) and are assumed to be competitive. Methods for measurement of larval competitive ability followed Kraaijeveld and Godfray (1997). The agar lined vials with 0.05 ml of yeast medium (25 g yeast per 100 ml water) were prepared. Twenty two-day old larvae of each experimental population were introduced

into a vial with 20 two-day old *D. simulans* larvae as tester flies, and emergence of *D. bipectinata* and *D. simulans* was examined. Measurements were made with 10 replicates for each population. The competition index was calculated by the following formula, log ((*b*+1)/(*t*+1)), where *b* is the number of flies of each population and *t* is the number of tester flies.

Viability at low and high temperatures. Egg-to-adult viability was examined at temperatures of 16 and 31.5°C in the same way as described above. These temperatures are close to lower and upper limits for the egg-to-adult development of *D. bipectinata*, respecitively (see Results).

Cold and heat tolerance. Larval and adult survival was examined at low and high temperatures that occur in the habitats or distribution range of the study species. To examine larval tolerance, three-day-old larvae were introduced into new vials with Drosophila medium, exposed to 10.5 or 35.5°C for 24 h, and then placed at 23°C. The number of flies that emerged from these vials was examined. Measurements were made with two replicates, each with approximately 50 individuals from each population. To examine adult tolerance, 7 to10-day old adult flies were placed at 7.5 or 34°C for 24 h, then placed at 23°C for 24 h for recovery, and examined for survival. Flies that were able to walk were assigned as survivors. Measurements were made with two replicates, each with approximately 20 individuals for each sex from each population.

Starvation tolerance. Adult flies (7-10 days after eclosion) were introduced into vials with non-nutritional medium containing only agar and water and placed under a continuous light at 23°C. Survivors were counted every 6 hours. Measurements were

made with eight replicates, each with approximately 10 individuals for each sex from each population.

Desiccation tolerance. Adult flies (7-10 days after eclosion) were introduced into empty vials covered with nylon gauze and placed in a desiccator (25×25×37 cm) with fresh silica gel under a continuous light at 23°C. In the desiccator, humidity fell below 10% within 1 h and gradually decreased further. Survivors were counted every 30 min. Measurements were made with two replicates, each with approximately 20 individuals for each sex from each population.

Resistance against other parasitoids

Resistance/susceptibility of the original populations and the selected and control populations against *L. victoriae* BG, *L. ryukyuensis* Novković & Kimura IR and *Asobara pleuralis* (Ashmead) KK was examined by parasitism experiments. *L. victoriae* BG originated from several females collected from Bogor in June 2008; *L. ryukyuensis* IR from those collected from Iriomote-jima in March 2005; *A. pleuralis* KK from those collected from Kota Kinabalu in March 2008. These parasitoid populations were maintained under 15L: 9D (15 h light: 9 h dark) at 23°C in laboratory for a few years using *D. simulans* as host. For the selected and control populations, flies of the eighth or ninth generation of the selection/control treatments were used. Parasitized larvae were prepared as explained previously (50 larvae for each population or each population), and the number of emergent flies or wasps was examined.

Data analysis

Longevity, starvation and desiccation tolerance were analyzed by survival analysis using “survival” package (Therneau and Lumley 2014) in R software version 2.15.1 (R Development Core Team, 2012). All survival models include the populations as a predictor variable and statistical significance of the predictor variable was obtained using log-lank test. We conducted post-hoc multiple comparisons for the traits that were shown to have significant effect of predictor variable. Significance levels among populations were corrected by Holm’s method (Holm 1979).

Other measured life history and stress tolerance traits were analyzed by fitting the generalized liner models (GLMs) using maximum likelihood in R. All GLMs include the populations as a predictor variable. To test statistical significance of the predictor variable, we calculate difference between -2 log likelihood of the model and null model using likelihood ratio test (LRT). For the traits that were shown to have significant effect of predictor variable, post-hoc multiple comparisons were conducted to assess the difference among the populations. Multiple comparisons among the populations of egg-to-adult development time and thorax length were carried out using the “multcomp” package (Hothorn et al. 2013) in R. Viability, heat and cold tolerance was analyzed by Fisher's exact test with correction of significance levels by Holm’s method. The three original populations and the experimental (selected and control) populations were analyzed independently in all life history and stress tolerance traits.

Resistance against other parasitoid population or species was analyzed by fitting the GLMs, and differences among populations were analyzed using LRT.

Results

Response to selection

The resistance of the base population (Generation 0 in Fig.1) against *L. victoriae* KK was low (resistance index: 0.15). The selected populations rapidly increased the resistance, and the resistance index reached 0.80 within four generations in the two replicate populations (Fig. 1). Resistance did not fall for at least 10 generations after selection was stopped at the 10th generation. In the two control populations, the resistance remained at low levels (resistance index: 0.1-0.3) except the second generation.

Life history and stress tolerance traits

Most life history and stress tolerance traits varied among the three original populations (Table 1). Consistent significant differences between resistant (BG and KK) and susceptible (IR) populations were observed in egg-to-adult development time, female longevity, egg-to-adult viability at 16 °C, male heat tolerance, starvation tolerance and female desiccation tolerance.

Among the selected and control populations, significant differences were observed in 11 traits; i.e., female and male longevity, female thorax length, female and male starvation tolerance, female desiccation tolerance, female and male adult heat tolerance, larval survival at heat and egg-to-adult viability at 23 and 31.5 °C (Table 2). Among these traits, only female longevity was lower in both selected populations compared with the two control populations, and thorax length was shorter and female desiccation tolerance and adult heat tolerance were lower at least in one of the selected populations compared with both or either of the control populations. In contrast, male longevity, male starvation tolerance and larval heat tolerance were higher in the selected populations compared with both or either of the control populations. Female starvation tolerance and egg-to-adult viability showed no distinct trend.

Resistance against other parasitoids

All of the original geographic populations and the selected and control populations were highly resistant to *L. victoriae* BG, a less virulent population compared with *L. victoriae* KK that was used in the selection experiment (Table 3). These populations also had resistance against *L. ryukyuensis* IR (Table 3). On the other hand, resistance against *A. pleuralis* KK significantly varied among the populations (LRT: $\chi^2 = 57.3$, $df = 6$, $P < 0.001$): *D. bipectinata* BG was rather resistant and *D. bipectinata* KK was slightly resistant, while *D. bipectinata* IR and the selected and control populations were almost susceptible. Among the selected and control populations, no significant difference was

observed in the resistance against *A. pleuralis* KK (LRT: $\chi^2 = 3.9$, $df = 3$, $P = 0.270$).

Discussion

The base population was constructed from two geographic (BG and KK) populations resistant against *L. victoriae* KK and one susceptible (IR) population. If each original population equally contributes to the genetic constitution of the base population, the base population would have a rather high resistance. However, it showed a relatively low resistance. The BG and KK populations may have possessed some low-fitness genes with which the resistance gene(s) are linked, and then the resistance may have been lowered in the base population before the linkage between these genes has been broken by recombination. Indeed, both or either of the BG and KK populations showed slower development, lower viability at 23 °C and shorter female longevity than the IR population, although male longevity was longer in the KK population compared with the IR population and fecundity and larval competitive ability did not significantly differ among these three populations (Table 1).

The selected populations rapidly increased resistance and became highly resistant to *L. victoriae* KK within four generations. This may suggest that the number of genes responsible for the difference in resistance between the IR population and the BG or KK populations is few. Indeed, simple genetic control of parasitoid resistance has also been reported in *D. melanogaster* and *D. yakuba* Burla (Carton et al. 1992; Kraaijeveld and van Alphen 1995; Dupas *et al.* 1998, 2003, 2009; Dubuffet et al. 2007,

2009).

In the present study, only female longevity was reduced in both selected (resistant) populations compared with the two control (susceptible) populations, but male longevity showed an opposite trend. On the other hand, female longevity did not significantly differ between the original resistant (BG and KK) and susceptible (IR) populations, and male longevity was low not only in the IR population but also in the BG population. Among the other traits, female desiccation tolerance and adult heat tolerance were reduced in one of the two selected populations. Female desiccation tolerance was also lower in the BG and KK populations compared with the IR population, but adult heat tolerance was much higher in the BG population compared with the KK and IR populations. Thus, the resistance against *L victoriae* KK may incur some costs to *D. bipectinata*, but it would not be high. This notion is supported by the present selection experiments where the resistance changed little for 10 or 20 generations in the selected and control populations if there was no artificial selection. However, it is still possible that the differences between the selected and control population is attributable to random drift, since the number of individuals used to produce the next generation was not large (i.e., 20-25) in the first few generations of selection. In addition, there may be some costs that cannot be detected by such laboratory experiments.

In previous selection experiments using *D. melanogaster*, a trade-off was observed between larval competitive ability and resistance against *L. boulardi* and *A. tabida* (Kraaijeveld and Godfray 1997; Fellowes et al. 1998). In the present study,

however, no significant difference was observed in competitive ability between the selected and control populations. This may be attributable to the difference in the drosophilid and parasitoid species studied or the difference in the type of competition; the previous studies examined intraspecific competition, whereas this study examined interspecific competition.

Irrespective of susceptibility/resistance against *L. victoriae* KK, all populations were resistant to *L. victoriae* BG and *L. ryukyuensis* IR, and all excepting the BG population were almost susceptible to *A. pleuralis*. Such parasitoid–species–specificity in resistance has been reported in a number of *Drosophila* species, and parasitoid–population–specificity has also been observed in some species (Dupas et al. 1998, 2003, 2009; Dubuffet et al. 2007, 2009; Mitsui and Kimura 2010; Novković et al. 2012; Kimura and Suwito 2014). If resistance is thus parasitoid–species–specific, host *Drosophila* species would be required to evolve a number of different resistance mechanisms, because they usually encounter a number of parasitoid species in nature (Mitsui and Kimura 2010; Novković et al. 2012; Kimura and Suwito 2012). Indeed, *D. bipectinata* from Iriomote-jima is resistant against *L. victoriae* BG, *L. ryukyuensis* and *Asobara japonica* Belokobylskij, and probably to *L. pacifica* Novković & Kimura (Novković et al. 2012). The low-cost nature of resistance may be important for host species to cope with a number of different parasitoid species.

Drosophila bipectinata is widely distributed in tropical Asia, and Iriomote-jima is located near the northern boundary of its distribution (Bock and Wheeler 1972; Lemeunier et al. 1986; Kopp and Barmia 2005). This species is cold

susceptible and its population in Iriomote-jima suffers high mortality in winter (Hirai et al. 2000; Kimura 2004; Novković et al. 2012). These things suggest that *D. bipectinata* has originated in the tropical regions of Asia and colonized Iriomote-jima rather recently. *Leptopilina victoriae* showed a similar distribution with *D. bipectinata*, but it occurs very rarely or only sporadically in Iriomote-jima (Nordlander 1980; Novković et al. 2011, 2012). It is therefore assumed that *D. bipectinata* in Iriomote-jima has lost resistance to *L. victoriae* KK, possibly as a result of low parasitism intensity (Novković et al. 2012). However, it is not known why *D. bipectinata* in Iriomote-jima still maintains resistance against *L. victoriae* from Bogor.

In conclusion, the resistance of *D. bipectinata* against *L. victoriae* KK probably incurs low fitness costs and specific to certain parasitoid populations or species (also see Dupas and Boscarl 1999; Kraaijeveld *et al*. 2001a; Dupas *et al*. 2009; Mitsui and Kimura 2010; Novković *et al*. 2012). In general, *Drosophila*-parasitoid systems are multispecific, i.e., a host species is parasitized by more than one parasitoid species, and a parasitoid species parasitizes more than one host species (Dupas *et al*. 2009; Mitsui and Kimura 2010; Novković *et al*. 2012; Kimura and Suwito 2012). If virulence and resistance are specific to a certain antagonist, such multispecific systems are possible only when virulence and resistance incur low costs; if a resistance to a parasitoid species is costly, it would be difficult to acquire resistance against a number of parasitoids. For further understanding of parasitoid-host associations, thus, it is important to assess the cost and specificity of virulence and resistance. One of important approaches to address this issue is identification of virulence and resistance genes by

quantitative trait loci analysis using AFLP or next-generation sequencers.

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Table 1. Mean ± SE (n) for life history and stress tolerance traits in three original populations and results of statistical analysis in three original populations. Different letters indicate statistically significant difference ($P < 0.05$) in post hoc multiple comparison.

Traits	Sex	Population			χ^2	df	P value
		RC	KK	IP			
Egg-to-adult development time (h)	Female	228.8 ± 1.12 (45)b	236.7 ± 1.36 (40)c	224.4 ± 0.48 (55)a	64.2	2	**< 0.0001**
	Male	233.7 ± 1.23 (61)b	238.9 ± 1.31 (37)c	228.7 ± 0.89 (54)a	31.4	2	**< 0.0001**
Egg-to-adult viability at 23.0 °C (%)	-	84.8 (125)b	61.6 (125)a	87.2 (125)b	27.8	2	**< 0.0001**
Longevity (day)	Female	36.4 ± 1.28 (90)a	35.3 ± 1.72 (79)a	40.8 ± 1.72 (111)b	12.8	2	**0.00167**
	Male	51.1 ± 1.82 (88)a	62.4 ± 2.56 (63)b	52.2 ± 1.49 (84)a	23.4	2	**< 0.0001**
Female fecundity (No. of egg)		293.6 ± 19.52 (21)	215.8 ± 32.59 (21)	247.0 ± 29.38 (21)	0.8	2	0.6782
Thorax length (um)	Female	888.3 ± 4.52 (30)a	934.6 ± 4.86 (30)b	902.5 ± 4.17 (30)a	44.0	2	**< 0.0001**
	Male	773.3 ± 3.92 (30)a	795.4 ± 4.46 (30)b	776.3 ± 4.42 (30)a	15.0	2	**0.0006**
Larval competitive ability (Index)		0.17 ± 0.060 (10*)	0.38 ± 0.077 (10*)	0.07 ± 0.023 (10*)	5.0	2	0.0831
Egg-to-adult viability at 16.0 °C (%)	-	0.0 (100) a	8.0 (100) b	22.0 (100) c	33.9	2	**< 0.0001**
Egg-to-adult viability at 31.5 °C (%)		5.0 (100)	13.0 (100)	7.0 (100)	4.4	2	0.1112
Larval survival at 10.5 °C (%)		15.0 (100) b	2.0 (100) a	15.0 (100) b	15.0	2	**0.0006**
Larval survival at 35.5 °C (%)	-	48.0 (100) b	27.0 (100) a	50.0 (100) b	13.8	2	**0.0010**
Adult survival at 7.5 °C (%)	Female	80.8 (52) b	23.9 (43) a	20.9 (46) a	47.8	2	**< 0.0001**
	Male	65.9 (41) b	16.7 (42) a	2.7 (37) a	45.2	2	**< 0.0001**
Adult survival at 34.0 °C (%)	Female	93.8 (48) b	51.2 (41) a	69.4 (49) a	22.8	2	**< 0.0001**
	Male	89.5 (38) c	50.0 (36) b	22.7 (44) a	40.6	2	**< 0.0001**
Survival time under starvation (h)	Female	70.8 ± 1.87 (53)b	76.4 ± 2.21 (52)b	58.2 ± 1.55 (49)a	45.7	2	**< 0.0001**
	Male	62.6 ± 2.30 (28)c	58.1 ± 1.06 (50)b	31.9 ± 0.87 (37)a	116.0	2	**< 0.0001**
Survival time under desiccation (h)	Female	4.03 ± 0.113 (43)b	3.60 ± 0.118 (46)a	5.04 ± 0.129 (42)c	47.3	2	**< 0.0001**
	Male	3.69 ± 0.140 (42)	3.44 ± 0.152 (42)	3.32 ± 0.110 (43)	4.2	2	0.123

3 *Number of vials. See method of larval competitive ability.

Table 2. Mean ± SE (n) for life history and stress tolerance traits in selected and control populations and results of statistical analysis.

Different letters indicate statistically significant difference ($P < 0.05$) in post hoc multiple comparison.

		Control populations		Selected populations				
Traits	**Sex**	**C1**	**C2**	**S1**	**S2**	**χ^2**	**df**	**P value**
Egg-to-adult development time (h)	Female	223.2 ± 0.84 (50)	224.0 ± 0.70 (47)	224.0 ± 0.82 (40)	222.0 ± 0.78 (54)	5.0	3	0.1191
	Male	229.3 ± 1.07 (50)	228.4 ± 0.98 (34)	230.1 ± 1.09 (20)	230.4 ± 1.22 (37)	1.9	3	0.5944
Egg-to-adult viability at 23.0 °C (%)	-	80.0 (125) a	64.8 (125) bc	55.2 (125) c	72.8 (125) ab	19.8	3	**0.0002**
Longevity (day)	Female	51.0 ± 2.03 (107) a	45.7 ± 2.10 (90) ab	39.3 ± 3.08 (46) bc	37.9 ± 1.95 (73) c	22.6	3	**< 0.0001**
	Male	59.8 ± 2.40 (69) b	56.2 ± 2.61 (61) b	73.6 ± 2.69 (34) a	71.6 ± 2.19 (57) a	20.2	3	**0.0002**
Female fecundity (No. of egg)		360.9 ± 32.39 (21)	364.7 ± 21.36 (21)	336.1 ± 28.15 (21)	304.4 ± 23.50 (21)	2.2	3	0.5418
Thorax length (μm)	Female	924.2 ± 5.36 (30) b	937.1 ± 3.81 (30) ab	941.3 ± 4.24 (30) a	937.1 ± 4.50 (30) ab	8.1	3	**0.0441**
	Male	792.5 ± 3.21 (30)	797.1 ± 3.73 (30)	803.6 ± 5.06 (28)	793.8 ± 4.75 (30)	4.1	3	0.2517
Larval competitive ability (Index)		0.24 ± 0.072 (10*)	0.22 ± 0.031 (10*)	0.35 ± 0.069 (10*)	0.17 ± 0.056 (10*)	1.3	3	0.7339
Egg-to-adult viability at 16.0 °C (%)	-	18.0 (100)	20.0 (100)	14.0 (100)	17.0 (100)	1.3	3	0.7212
Egg-to-adult viability at 31.5 °C (%)		9.0 (100) ab	22.0 (100) a	13.0 (100) b	26.0 (100) ab	13.2	3	**0.0042**
Larval survival at 10.5 °C (%)		20.0 (100)	22.0 (100)	19.0 (100)	15.0 (100)	1.7	3	0.6303
Larval survival at 35.5 °C (%)	-	61.0 (100) b	67.0 (100) a	34.0 (100) a	56.0 (100) a	25.3	3	**< 0.0001**
Adult survival at 7.5 °C (%)	Female	36.4 (44)	54.5 (44)	35.9 (39)	53.8 (39)	5.5	3	0.1380
	Male	6.7 (45)	10.5 (38)	0.0 (36)	8.3 (36)	5.9	3	0.1172
Adult survival at 34.0 °C (%)	Female	76.7 (43) a	66.7 (42) ab	22.9 (35) c	46.3 (41) bc	27.4	3	**< 0.0001**
	Male	40.0 (40) a	38.6 (44) ab	13.2 (38) b	29.5 (44) ab	9.2	3	**0.0262**
Survival time under starvation (h)	Female	57.9 ± 1.49 (80)ab	62.3 ± 1.33 (84)a	57.8 ± 1.17 (76)b	60.6 ± 1.31 (67)ab	7.9	3	**0.0482**
	Male	36.3 ± 0.77 (93) b	37.4 ± 0.93 (81) b	40.8 ± 0.91 (100) a	41.3 ± 0.77 (106) a	24.3	3	**< 0.0001**
Survival time under desiccation (h)	Female	5.38 ± 0.122 (40) a	5.33 ± 0.196 (39) ab	4.87 ± 0.123 (45) b	5.55 ± 0.187 (44) a	14.9	3	**0.00189**
	Male	3.59 ± 0.137 (43)	3.57 ± 0.110 (41)	3.61 ± 0.116 (43)	3.99 ± 0.149 (39)	5.8	3	0.124

6 *Number of vials. See method of larval competitive ability.

Table 3. Results of parasitism by *L. victoriae* BG, *L. ryukyuensis* IR and *A. pleuralis* KK in the original (BG, KK and IR) populations and the selected (S1 and S2) and control populations (C1 and C2) of *D. bipectinata*.

	L. victoriae BG			*L. ryukyuensis* IR			*A. pleuralis* KK		
Population	F	W	D	F	W	D	F	W	D
BG	41	0	9	30	0	20	17	11	22
KK	39	0	11	38	0	12	5	31	14
IR	35	0	15	38	0	12	1	33	16
C1	45	0	15	30	0	20	3	34	13
C2	39	1	10	41	0	9	1	31	18
S1	37	0	13	39	0	11	1	39	10
S2	45	0	5	42	0	8	0	29	21

F: number of flies that emerged, W: number of wasps that emerged, D: number of host larvae from which neither fly nor wasp emerged. No significant difference was observed in resistance against the three parasitoid strains at least among the selected and control lines (LRT, $P<0.05$).

Figure legends

Figure 1. Response to selection. Control populations (C1; open circle, C2; open square) and selected populations (S1; closed circle, S2; closed square). Selected populations were maintained without selection after 10th generation (indicated by arrow).