

Effects of intraspecific hybridization on the fitness of the egg parasitoid *Trichogramma galloi*

Aline Bertin · Vitor Antonio Corrêa Pavinato · José Roberto Postali Parra

Received: 5 March 2018 / Accepted: 16 April 2018 / Published online: 19 April 2018
© International Organization for Biological Control (IOBC) 2018

Abstract Successive rearing in laboratory conditions can result in the loss of genetic diversity, inbreeding depression and adaptation to the captive environment, affecting the quality of the insects reared and compromising their field performance. Introduction of genetic variation by admixing different populations may increase the fitness of populations, minimizing the negative effects of rearing many generations in artificial conditions. We experimentally investigated the role of intraspecific hybridization in enhancing the fitness of the egg parasitoid *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae), by reciprocally crossing three populations. Our results showed that the mating type did not affect the number of crosses that produced viable daughters. Homotypic crosses produced 94% viable daughters, while heterotypic crosses produced

92%. There were neither mating incompatibilities nor reproductive barriers between these populations. However, we observed a low fitness value for females from one of the populations studied. The fitness of hybrids was either unchanged or improved (in one case) when compared to the parental populations. We discuss the implications of our results and suggest future research directions.

Keywords Intraspecific hybridization · Heterosis · Fitness · Biological control · Hymenoptera · Trichogrammatidae

Introduction

Laboratory and mass rearing of biological control agents can have detrimental effects on insect performance. Successive rearing in laboratory conditions

Handling Editor: Stefano Colazza.

A. Bertin · J. R. P. Parra
Department of Entomology and Acarology, University of São Paulo, Avenida Pádua Dias 11, CP 9, Piracicaba, SP 13418-900, Brazil
e-mail: jrpparra@usp.br

V. A. C. Pavinato
Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University, Thorne Hall, 1680 Madison Avenue, Wooster, OH, USA
e-mail: vitor.pavi@gmail.com

Present Address:
V. A. C. Pavinato
Institute National de la Recherche Agronomique – INRA, Centre de Biologie pour la Gestion des Populations – CBGP, 755 Avenue du Campus Agropolis, CS 30016, 34988 Montpellier-sur-Lez Cedex, France

A. Bertin (✉)
Department of Entomology and Acarology, University of São Paulo – ESALQ/USP, Avenida Pádua Dias 11, CP 9, Piracicaba, SP 13418-900, Brazil
e-mail: aline.bertin@usp.br

can result in the loss of genetic diversity, inbreeding depression and adaptation to the captive environment (Frankham 2008; Henter 2003; Hopper et al. 1993; Woodworth et al. 2002), which subsequently can affect the quality of the insects reared and compromise the success of biological control programs. To mitigate these effects, introduction of genetic variation by admixing different populations (intraspecific hybridization) can be a promising strategy.

Cross-breeding between divergent populations may increase the fitness of the hybrid offspring (heterosis) and/or increase the genetic variance of phenotypic traits (Benvenuto et al. 2012; Facon et al. 2011; Szücs et al. 2012), facilitating evolution and response to new environmental pressures (Anderson and Stebbins 1954; Facon et al. 2008, 2011; Lewontin and Birch 1966; Rius and Darling 2014) and alleviating the negative effects of inbreeding and drift (Whitlock et al. 2000). Hybrids with higher fitness will produce a large number of new combinations of traits and genotypes in the early generations, increasing the chance that some recombinants may better adapt to a novel environment, and thus be favored by natural selection (Johansen-Morris and Latta 2006). Consistently with these expectations, many empirical studies have found an increase in the vigor of the progeny of crosses between different populations, as is the case for many biological control agents (Benvenuto et al. 2012; Seko et al. 2012; Szücs et al. 2012).

Despite its benefits, hybridization can negatively impact the fitness of first-generation hybrids (F1) due to genetic incompatibilities, disruption of local adaptations (gene \times environment interactions), epistatic interactions, or underdominance (Burke and Arnold 2001; Edmands 2007; Lynch and Walsh 1998), resulting in outbreeding depression. Outbreeding depression is likely to occur in crosses between divergent populations. Although less empirical evidence is available than for inbreeding depression, outbreeding depression has been recognized in both plants and animals (e.g., Andersen et al. 2002; Waser et al. 2000), including biological control agents (Benvenuto et al. 2012; Vorsino et al. 2012).

Although the outcomes of hybridization are difficult to predict, biological control operations could benefit greatly from the positive outcomes. An increase in fitness or genetic variance could, in the case of biological control agents, mean increased population efficiency and persistence in the field, and increased

viability of laboratory populations suffering the effects of genetic drift and inbreeding (Seko et al. 2012).

Given that species of *Trichogramma* are widely used in biological control programs (Hassan 1993; Parra and Zucchi 2004) and are easy to handle and maintain in laboratory conditions, they can be ideal candidates to experimentally investigate the effects of intraspecific hybridization on the fitness of biological-control agents. In this study, we reciprocally crossed three populations of the egg parasitoid *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae), which is extensively used to control the sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) (Parra and Zucchi 2004; Parra 2014), to investigate the outcome of intraspecific hybridization in a species that has proven to be negatively affected by laboratory rearing conditions (Bertin et al. 2017). The ultimate goal of this study was to evaluate the potential use of intraspecific crosses in biological control programs, in an attempt to increase the fitness of biological control agents and mitigate the negative effects of mass rearing.

Materials and methods

Trichogramma galloi populations

In this experiment, we used three populations that were originally collected in different locations in Brazil: two populations from Minas Gerais state, one from Santa Vitoria (SV) and another from an unknown location (here designated MG); and one population from Bahia state (BA). We maintained these populations on eggs of the factitious host *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) (Parra, 1997) under standard conditions (25 ± 1 °C, $70 \pm 10\%$ RH and a L:D 14:10 photoperiod) for more than 20 generations.

Detection of *Wolbachia* infections in populations of *Trichogramma galloi*

We tested for the presence of *Wolbachia* in individuals of each population prior to the intraspecific crosses. This endosymbiont can cause reproductive barriers in arthropods (Engelstädter and Hurst 2009; Stouthamer et al. 1999), making it important to identify potential infections, as they can cause mating incompatibilities

that are independent of the genetic background of the population. For the DNA extraction, we randomly collected individuals from each population and placed them in 1.5-ml tubes containing 80 μl of 10% Chelex and 8 μl of proteinase K (20 mg ml⁻¹). We macerated each individual with a sterilized pestle and incubated it at 95 °C in a heat block for 20 min. Then, each sample was centrifuged and the supernatant was recovered. To the supernatant, we added 20 μg of glycogen, 0.6 volume of isopropanol and 0.1 volume of 3 M sodium acetate (pH 5.2) to precipitate the DNA. The samples were incubated at - 80 °C for 30 min and then centrifuged (17,000 $\times g$) for 30 min. The supernatant was discarded and the DNA pellet washed in successive baths of 85% ethanol and centrifuged (17,000 $\times g$) for 10 min. The DNA pellet was maintained at 65 °C for approximately 5 min to complete the ethanol evaporation. We added 10 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to each sample and incubated it at 37 °C for 30 min. All samples were stored at - 20 °C prior to use.

The presence of *Wolbachia* in *T. galloi* populations was detected by amplification of a fragment of the *wsp* gene, which codes for a specific cell wall protein of this symbiont, using the specific primers *wsp* 81F (5'-TGGTCCAATAAGTGATGAAGAAAC-3') and *wsp* 691R (5'-AAAAATTAACGCTACTCCA-3'), in a PCR reaction programmed at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min (35 cycles), followed by final extension at 72 °C for 5 min (1 cycle) (Braig et al. 1998). The reactions were performed in a total volume of 25 μl , containing 1 μl of gDNA, 1 \times PCR buffer, 1.5 mM MgCl₂, 200 μM of each dNTP, 0.32 mM of each primer and 0.5 U of Taq polymerase. The amplification products were separated by electrophoresis on a 1% agarose gel and visualized on a transilluminator (UV) coupled to the image capture and scanning system.

Negative samples were re-amplified in a nested reaction, with the primers *wsp* 106F (5'-GGA-TAGTCCCTTAACAAGAT-3') and *wsp* 460R (5'-TTGATTTCTGGAGTTACATC-3'), under identical conditions to those described above for the other set of primers. As positive controls, we used infected individuals of *Diaphorina citri* (Hemiptera: Liviidae).

Removal of *Wolbachia* from *Trichogramma galloi* populations

After detecting *Wolbachia* in both populations from Minas Gerais state, we randomly selected individuals from these populations to initiate *Wolbachia*-free strains. Individuals of each population were fed with pure honey plus an antibiotic (0.25% tetracycline) for three generations (Stouthamer et al. 1990). After three generations, we randomly selected females from both populations to perform PCR diagnoses, in order to confirm that the bacteria had been eliminated.

Intraspecific crosses

We used the three populations in a full diallel mating design, where the parents were tested in couples, in all possible combinations. The experimental design was replicated three times, for a total of 25 couples for each cross. We offered approximately 100 eggs (24 h old) of *E. kuehniella* to each couple for an oviposition period of 48 h, and evaluated the reproductive success by checking the presence of females in the progeny. The offspring were allowed to mate, and after 24 h we isolated two females from each cross in glass tubes containing eggs (24 h old) of *E. kuehniella* for an oviposition period of 48 h. We used the number of parasitized eggs per female as a fitness measure.

For each cross, we calculated the proportion of couples that produced at least one viable daughter. These values were analyzed using the generalized linear mixed-effects model (GLMM) with a binomial distribution and a logit link function, using the lme4 package of the R software (Bates et al. 2015). The fixed explanatory variable was the mating type (homotypic: both parents belonging to the same population; heterotypic: parents belonging to different populations) and the random variables: block and the geographical origin of the population.

We analyzed the number of parasitized eggs per female of the parental populations using a linear model, and compared the means using a Tukey test ($\alpha = 0.05$). We performed all analyses in the software R version 3.4.0 (R Development Core Team 2017).

Mating compatibilities between populations of *Trichogramma galloi*

To assess the degree of sexual isolation among populations, we used the I_{PSI} estimator proposed by Rolán-Alvarez and Caballero (2000). Estimated values can range from -1 (heterotypic mating pairs: when mating occurs only between parents of different origin) to 1 (homotypic mating pairs: when mating occurs only between parents of the same origin), with 0 corresponding to random mating (Carvajal-Rodríguez and Rolán-Alvarez 2006; Rolán-Alvarez and Caballero 2000). We also used the asymmetry index (IA_{PSI}) to quantify the asymmetry between matings (♀_x mated with ♂_y versus ♀_y mated with ♂_x), with values significantly different from 1 (symmetry) indicating asymmetry (Carvajal-Rodríguez and Rolán-Alvarez 2006). In addition, to measure the mating success of each population relative to the one with the highest sexual fitness (fitness 1) we used the 'cross-product estimator W ', for each sex separately. A bootstrap analysis (10,000 iterations) was used to calculate the SD of these parameters, as well as deviations from the threshold values (0 , 1 , and 1 , for I_{PSI} , IA_{PSI} and W , respectively). Overall, these estimates based on mating frequencies can help detect populations with low fitness and incompatibilities between the populations crossed. We estimated all indexes in the program JMATING (Carvajal-Rodríguez and Rolán-Alvarez 2006).

Fitness of *Trichogramma galloi* hybrids

We estimated the fitness of hybrids using three estimators: (i) best parent heterosis (BPH) defined as the difference between the average fitness of the hybrids and the average fitness of the higher-performing parent; (ii) mid parent heterosis (MPH) defined as the difference between the average fitness of the hybrids and the average fitness of the two parents; (iii) lowest parent heterosis (LPH) defined as the difference between the average fitness of the hybrids and the average fitness of the lower-performing parent. These estimates are used to evaluate the improvement of desired traits in plant and animal breeding. For practical applications, BPH is the most important. A positive BPH value indicates that the fitness of the hybrid is higher relative to the best parental population. Positive MPH and LPH values indicate that the

fitness of the hybrid is superior only when compared to the mean of the parental populations or the lower-performing parent, respectively. Negative values for these estimates indicate a decrease in the fitness of hybrids. We tested each hybrid against the higher-, mid- and lower-performing parents through t tests, to detect either heterosis or a reduction in fitness, using R version 3.4.0 (R Development Core Team 2017).

Results

Detection of *Wolbachia* infections in populations of *Trichogramma galloi*

We detected the presence of *Wolbachia*, in low frequencies, in two populations of *T. galloi* (MG and SV). In some individuals, the infection was detected only after re-amplification of the first PCR products in a nested reaction. These populations were then treated for three generations with pure honey plus antibiotic, until the complete elimination of the endosymbiont (confirmed by PCR reactions).

Intraspecific crosses among three populations of *Trichogramma galloi*

The number of viable daughters produced by each cross was not affected by the mating type ($\chi^2 = 0.1081$, $df = 1$, $P = 0.742$). Homotypic crosses produced a mean of 94% viable daughters, whereas heterotypic crosses resulted in a mean of 92% viable daughters. Among the homotypic crosses, the lowest percentage of successful matings was observed in the population $SV\text{♀}SV\text{♂}$ (52%), whereas for heterotypic crosses the lowest percentages were observed in the populations $SV\text{♀}MG\text{♂}$ (36%) and $SV\text{♀}BA\text{♂}$ (44%) (Table 1).

The number of parasitized eggs per female in the parental populations was significantly different ($F_{2,72} = 4.938$, $P = 0.009$). The number of parasitized eggs per female was higher in the populations from Bahia (BA, mean 35 parasitized eggs) and Minas Gerais (MG, mean 32 parasitized eggs), in contrast to the population from Santa Vitoria (SV, mean 19 parasitized eggs) (Fig. 1).

Table 1 Estimates of the coefficients for sexual isolation (I_{PSI}) and asymmetry index (IA_{PSI}) \pm SD for the different crosses of *Trichogramma galloi*

	SV ♂	MG ♂	BA ♂
SV ♀			
n (t)	13 (25)	9 (25)	11 (25)
I_{PSI}		0.05 \pm 0.13	0.06 \pm 0.13
IA_{PSI}		0.94 \pm 0.13	0.95 \pm 0.09
MG ♀			
n (t)	21 (25)	18 (25)	20 (25)
I_{PSI}			0.01 \pm 0.11
IA_{PSI}			1.00 \pm 0.03
BA ♀			
n (t)	20 (25)	21 (25)	22 (25)

The I_{PSI} and IA_{PSI} estimates for the different crosses did not deviate from the threshold values

n number of successful matings (producing viable daughters), *t* total number of crosses. SV Santa Vitória, MG; MG Minas Gerais; BA Bahia

Mating compatibilities among populations of *Trichogramma galloi*

The I_{PSI} and IA_{PSI} values used to detect sexual isolation and asymmetric mating compatibilities were not significant (Table 1). However, we observed a low fitness value (*W*, cross-product estimator) for females from the SV population (Table 2).

Fitness of *Trichogramma galloi* hybrids

The fitness of hybrids was either unchanged or improved when compared to the parental populations. When comparing the performance of F1 hybrids with the higher-performing parent, we did not observe heterosis (Table 3). However, we did observe positive heterosis over the mid- (34.58%) and lower-performing parents (88.38%) in one hybrid population ($BA_{♀}SV_{♂}$) (Table 3, Fig. 1). Other hybrids also presented positive heterosis over the mid- and lower-performing parents. However the values were not statistically significant (Table 3).

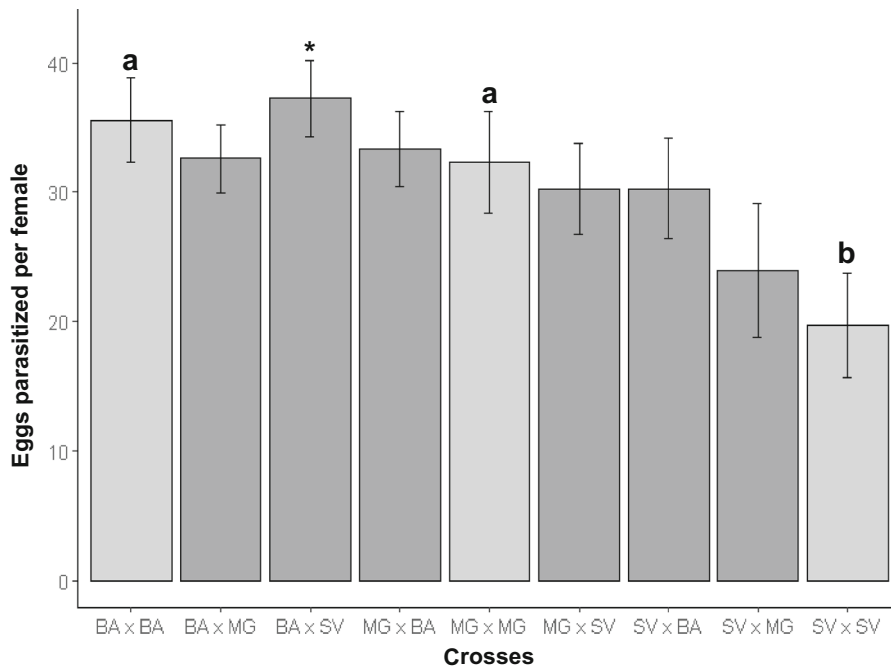


Fig. 1 Mean number of parasitized eggs per female (\pm SE) of the parental populations (light gray) and F1 hybrids (dark gray) of *Trichogramma galloi* reared on the factitious host *Ephesthia kuehniella* (SV Santa Vitória, MG; MG Minas Gerais; BA Bahia). In each cross (F \times M), the abbreviation on the left side

corresponds to females and on the right side to males. Differences in the mean number of parasitized eggs per female in the parental populations are indicated by lower-case letters ($P < 0.05$). The asterisk indicates the hybrid population that presented lowest and mid parent heterosis

Table 2 Cross-product estimates ($W \pm SD$) of females and males of *Trichogramma galloi* and corresponding P values (P)

Population	Females		Males	
	W	P	W	P
SV	0.53 ± 0.11	0.0008	1	
MG	0.95 ± 0.17	0.37	0.9 ± 0.18	0.29
BA	1		0.99 ± 0.19	0.47

Single values are tested against the reference value ($W = 1$) using a bootstrap analysis

SV Santa Vitória, MG; MG Minas Gerais; BA Bahia

Discussion

Introduction of genetic variation has proved to effectively increase the fitness of laboratory populations (Hoy 1976; Seko et al. 2012). Introduction of external variation can minimize the negative effects of inbreeding depression (Pekkala et al. 2012) and increase the genetic variation, which may facilitate adaptation to new environmental pressures (Anderson and Stebbins 1954; Facon et al. 2008, 2011; Lewontin and Birch 1966). By conducting intraspecific crosses and measuring the number of parasitized eggs per female of the egg parasitoid *T. galloi*, our study showed that hybridization had little or no effect in increasing the fitness of our laboratory populations.

The W estimator allowed us to detect a low fitness value for females of the SV population. In addition, the number of parasitized eggs per female of the SV parental population was significantly lower than that of other parental populations. The reduced number of parasitized eggs observed in this population may be

related to the successive rearing in laboratory conditions, which could reduce fitness due to the loss of genetic diversity, adaptation to the captive environment or inbreeding depression. Although it is recognized that haplodiploid species should be less sensitive to inbreeding given that recessive deleterious alleles that are expressed in haploid males are purged through selection, alleles of genes only expressed in the diploid females are protected in heterozygous individuals (Antolin 1999; Werren 1993). Therefore, haplodiploids should suffer more from inbreeding effects on life-history traits controlled by genes with female-limited expression (Saito et al. 2000; Tien et al. 2015). However, with our experimental design we are not able to confirm if the low parasitism rate of the SV parental population is due to inbreeding depression, as another experimental design should be used to confirm this hypothesis. In addition, the low parasitism rate of the SV parental population could be also determined by a low fecundity of females that were the origin of this strain.

The fitness of hybrids was either unchanged or improved (in one case) with hybridization when compared to the parental populations. The hybrid $BA_{\text{♀}}SV_{\text{♂}}$ had an increase of approximately 35 and 89% in the number of parasitized eggs per female, compared with the average of its mid- (average of 27 parasitized eggs) and lower-performing parent (average of 19 parasitized eggs), respectively. This hybrid population may have benefited from an increase in genetic variation, either by new or recombinant genotypes or by the masking of deleterious mutations. Although significant, these results have less practical

Table 3 Lowest parent heterosis (LPH), mid parent heterosis (MPH) and best parent heterosis (BPH) in *Trichogramma galloi* hybrids and corresponding P values (P)

Cross	LPH				MPH				BPH			
	(%)	t	df	P	(%)	t	df	P	(%)	t	df	P
$SV_{\text{♀}} \times BA_{\text{♂}}$	53.20	1.866	45	0.068	9.44	0.526	70	0.600	-14.86	-1.045	45	0.301
$SV_{\text{♀}} \times MG_{\text{♂}}$	21.15	0.644	40	0.522	-8.05	-0.359	65	0.720	-25.92	-1.314	40	0.196
$BA_{\text{♀}} \times MG_{\text{♂}}$	0.77	0.055	65	0.955	-4.03	-0.375	90	0.708	-8.40	-0.710	65	0.479
$BA_{\text{♀}} \times SV_{\text{♂}}$	88.38	3.576	63	< 0.001*	34.58	2.337	88	0.021*	4.68	0.367	63	0.714
$MG_{\text{♀}} \times SV_{\text{♂}}$	53.14	1.916	65	0.059	16.21	0.936	90	0.351	-6.36	-0.379	65	0.705
$MG_{\text{♀}} \times BA_{\text{♂}}$	3.16	0.212	61	0.832	-1.76	-0.154	86	0.877	-6.23	-0.492	61	0.623

SV Santa Vitória, MG; MG Minas Gerais; BA Bahia)

*Indicate statistical significant results ($p < 0.05$)

value for the optimization of biological control agents when compared to the best parent heterosis (BPH).

On the other hand, we did not observe heterosis relative to the better parents, as the difference between the average fitness of the hybrids and the average fitness of the higher-performing parents was not significant. This result suggests that these three populations have a low level of genetic divergence, which did not lead to new beneficial phenotypes relative to the better parental populations. Hybridization is expected to have minimal effects on fitness, particularly when the genetic distance of the mixing populations is small (Mallet 2005). Future studies could incorporate molecular markers to estimate the genetic diversity and genetic distance between parental populations before performing intraspecific crossings. This could help us define which populations to cross in order to increase the chances of obtaining positive values of heterosis.

Our results also provided no evidence for a reduction in the performance of the hybrids: no mating incompatibilities or reproductive barriers were observed that could compromise the use of intraspecific crosses to improve the fitness of this biological-control agent. These results indicate that hybridization can also have neutral effects, with no direct impact on phenotypic traits. The effects of hybridization can be beneficial, neutral, or harmful, and they depend on a number of factors, including the level of genetic divergence among populations, the amount of genetic variation introduced, the environmental conditions, the traits being evaluated, and the number of generations after hybridization (Burke and Arnold 2001; Edmands 2007; Pekkala et al. 2012).

Because of the many factors that may influence crosses, it is difficult to define a safe level of divergence for interpopulation hybridization. Given this degree of unpredictability, the data obtained for a particular cross cannot predict the consequences of other crosses. However, genetic distance, in addition to phenotypic distance, may help to determine the fitness of F1 hybrids (Benvenuto et al. 2012). Genetic distance can also help to better define the degree of incompatibility between populations (Benvenuto et al. 2012; Edmands 2002): a positive relationship between the genetic distance and the degree of incompatibility of populations was observed by Benvenuto et al. (2012). Unfortunately, there are still no alternative ways to test the direct effects of hybridization without

performing crosses in the laboratory. Studies involving phenotypic, geographic and genetic distances may help to unravel the effects of hybridization and increase the chances of predicting the fitness of hybrids, thus increasing the success of biological pest control strategies. In addition, quantitative genetic and population genomic methods could be applied in the selective breeding of biological control agents (Lommen et al. 2017; Wright and Bennett 2018). With the advance of our current knowledge and technologies in these scientific fields, we could develop genome selection methods to improve relevant traits. Finally, it would be important to evaluate the field performance of the hybrids to confirm if the benefits obtained in the laboratory persist after field release.

In the present study, intraspecific hybridization had little (one positive case of LPH and MPH) or no effect in increasing the fitness of laboratory populations of the egg parasitoid *T. galloi*. For the optimization of biological control agents, it would be important to obtain positive heterosis relative to the best parental population. Although we have not obtained positive results (of BPH) with this technique, we cannot discourage future research on this subject as the effects may vary according to the intrinsic characteristics of the populations, the genetic distance between parental populations and the traits being measured.

Acknowledgements We thank Dr. Janet W. Reid (JWR Associates) for English and technical corrections, the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Processo: 140539/2012-3) for granting a scholarship to the first author, and the National Institute of Semiochemicals in Agriculture (Process nos. CNPq 573761/2008-6 and FAPESP 2008/57701-2) for financial support.

Funding This study was funded by the National Institute of Semiochemicals in Agriculture (Process nos. CNPq 573761/2008-6 and FAPESP 2008/57701-2).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Andersen DH, Putridly C, Scali V, Loeschcke V (2002) Intraspecific hybridization, developmental stability and fitness in *Drosophila mercatorum*. *Evol Ecol Res* 4:603–621

- Anderson E, Stebbins GL (1954) Hybridization as an evolutionary stimulus. *Evolution* 8:378–388
- Antolin MF (1999) A genetic perspective on mating systems and sex ratios of parasitoid wasps. *Res Popul Ecol* 41:29–37
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Benvenuto C, Tabone E, Vercken E, Sorbier N, Colombel E, Warot S, Fauvergue X, Ris N (2012) Intraspecific variability in the parasitoid wasp *Trichogramma chilonis*: can we predict the outcome of hybridization? *Evol Appl* 5:498–510
- Bertin A, Pavinato VAC, Parra JRP (2017) Fitness-related changes in laboratory populations of the egg parasitoid *Trichogramma galloi* and the implications of rearing on factitious hosts. *BioControl* 62:435–444
- Braig HR, Zhou W, Dobson SL, O'Neil SL (1998) Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *J Bacteriol* 180:2373–2378
- Burke JM, Arnold ML (2001) Genetics and the fitness of hybrids. *Annu Rev Genet* 35:31–52
- Carvajal-Rodríguez A, Rolán-Alvarez E (2006) JMATING: a software for the analysis of sexual selection and sexual isolation effects from mating frequency data. *BMC Evol Biol* 6:40
- Edmunds S (2002) Does parental divergence predict reproductive compatibility? *Trends Ecol Evol* 17:520–527
- Edmunds S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol Ecol* 16:463–475
- Engelstädter J, Hurst GDD (2009) The ecology and evolution of microbes that manipulate host reproduction. *Annu Rev Ecol Syst* 40:127–149
- Facon B, Pointier JP, Jarne P, Sarda V, David P (2008) High genetic variance in life-history strategies within invasive populations by way of multiple introductions. *Curr Biol* 18:363–367
- Facon B, Crespin L, Loiseau A, Lombaert E, Magro A, Estoup A (2011) Can things get worse when an invasive species hybridizes? The harlequin ladybird *Harmonia axyridis* in France as a case study. *Evol Appl* 4:71–88
- Frankham R (2008) Genetic adaptation to captivity in species conservation programs. *Mol Ecol* 17:325–333
- Hassan SA (1993) The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: achievements and outlook. *Pestic Sci* 37:387–391
- Henter HJ (2003) Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evolution* 57:1793–1803
- Hopper KR, Roush RT, Powell W (1993) Management of genetics of biological control introductions. *Annu Rev Entomol* 38:27–51
- Hoy MA (1976) Genetic improvement of insects: fact or fantasy. *Environ Entomol* 5:833–839
- Johansen-Morris AD, Latta RG (2006) Fitness consequences of hybridization between ecotypes of *Avena barbata*: hybrid breakdown, hybrid vigor and transgressive segregation. *Evolution* 60:1585–1595
- Lewontin RC, Birch LC (1966) Hybridization as a source of variation for adaptation to new environments. *Evolution* 20:315–336
- Lommen STE, de Jong PW, Pannebakker BA (2017) It is time to bridge the gap between exploring and exploiting: prospects for utilizing intraspecific genetic variation to optimize arthropods for augmentative pest control—a review. *Entomol Exp Appl* 162:108–123
- Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland
- Mallet J (2005) Hybridization as an invasion of the genome. *Trends Ecol Evol* 20:229–237
- Parra JRP (1997) Técnicas de criação de *Anagasta kuehniella*, hospedeiro alternativo para produção de *Trichogramma*. In: Parra JRP, Zucchi RA (eds) *Trichogramma e o controle biológico aplicado*. FEALQ, Piracicaba, pp 121–150
- Parra JRP (2014) Biological control in Brazil: an overview. *Sci Agric* 71:420–429
- Parra JRP, Zucchi AR (2004) *Trichogramma* in Brazil: feasibility of use after twenty years of research. *Neotrop Entomol* 33:271–281
- Pekkala N, Knott KE, Kotiaho JS, Nissinen K, Puurtinen M (2012) The benefits of interpopulation hybridization diminish with increasing divergence of small populations. *J Evol Biol* 25:2181–2193
- R Development Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.r-project.org/>
- Rius M, Darling JA (2014) How important is intraspecific genetic admixture to the success of colonising populations? *Trends Ecol Evol* 29:233–242
- Rolán-Alvarez E, Caballero M (2000) Estimating sexual selection and sexual isolation effects from mating frequencies. *Evolution* 54:30–36
- Saito Y, Sahara K, Mori K (2000) Inbreeding depression by recessive deleterious genes affecting female fecundity of a haplo-diploid mite. *J Evol Biol* 13:668–678
- Seko T, Miyatake T, Miura K (2012) Assessment of hybrid vigor between flightless lines to restore survival and reproductive characteristics in the ladybird beetle *Harmonia axyridis*. *BioControl* 57:85–93
- Stouthamer R, Luck RF, Hamilton WD (1990) Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera: Trichogrammatidae) to revert sex. *Proc Natl Acad Sci USA* 87:2424–2427
- Stouthamer R, Breeuwer JAJ, Hurst GDD (1999) *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu Rev Microbiol* 53:71–102
- Szücs M, Eigenbrode SD, Schwarzländer M, Schaffner U (2012) Hybrid vigor in the biological control agent, *Longitarsus jacobaeae*. *Evol Appl* 5:489–497
- Tien NSH, Sabelis MW, Egas M (2015) Inbreeding depression and purging in a haplodiploid: gender-related effects. *Heredity* 114:327–332
- Vorsino AE, Wiecezorek AM, Wright MG, Messing RH (2012) An analysis of heterosis and outbreeding depression among lab-reared populations of the parasitoid *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae); potential implications for augmentative releases. *Biol Control* 61:26–31

- Waser NM, Price MV, Shaw RG (2000) Outbreeding depression varies among cohorts of *Ipomopsis aggregata* planted in nature. *Evolution* 54:485–491
- Werren JH (1993) The evolution of inbreeding in a haplodiploid organism. In: Thornhill NW (ed) *The natural history of inbreeding and outbreeding*. University of Chicago Press, Chicago, pp 42–59
- Whitlock MC, Ingvarsson PK, Hatfield T (2000) Local drift load and the heterosis of interconnected populations. *Heredity* 84:452–457
- Woodworth LM, Montgomery ME, Briscoe DA, Frankham R (2002) Rapid genetic deterioration in captive populations: causes and conservation implications. *Conserv Genet* 3:277–288
- Wright MG, Bennett GM (2018) Evolution of biological control agents following introduction to new environments. *BioControl* 63:105–116

Aline Bertin is an entomologist with experience in insect biology and biological control. She has particular interest in using recent scientific developments in population biology, ecology and genetics to improve the understanding and practice of biological control.

Vitor Antonio Corrêa Pavinato is a geneticist involved in research projects with special focus on molecular ecology and adaptation. He has experience with population genomics, statistical genetics and data analysis.

José Roberto Postali Parra is a full professor at the Department of Entomology and Acarology, College of Agriculture “Luiz de Queiroz” (ESALQ), University of Sao Paulo, Brazil and a Member of the Brazilian Academy of Sciences. He has extensive experience with insect biology, nutrition, and biological control, with emphasis on parasitic Hymenoptera, especially *Trichogramma*.