Behavioral Ecology

The official journal of the ISBE International Society for Behavioral Ecology

Behavioral Ecology (2014), 25(3), 553–564. doi:10.1093/beheco/aru015

Original Article

Host-shift effects on mating behavior and incipient pre-mating isolation in seed beetle

Biljana Stojković,[a,b] Uroš Savković,[b] Mirko Đorđević,[b] and Nikola Tucić[b]

[a]Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia and [b]Institute for Biological Research, Siniša Stanković, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia

Received 4 December 2013; revised 24 December 2013; accepted 13 January 2014; Advance Access publication 24 February 2014.

Mating behavior is based on communication among mates and includes both sexual signaling and mating preferences. In phytophagous insects, shift to a novel host will expose associated traits to novel selection regimes and eventually cause assortative mating and sexual isolation between populations inhabiting diverse host plants. We investigated the relationship between short- and long-term changes in mating systems on *Acanthoscelides obtectus*. Mating preferences were evaluated using measures of copulation frequency in mating trials within and among populations and by measuring time spent in sexual interactions prior to copulation. Sexual signaling was previously analyzed by chemical detection of contact pheromones (i.e., cuticular hydrocarbons [CHCs]), and these results were used in this study. Laboratory populations evolved for 50 generations either on the optimal host (common bean) or on the suboptimal host (chickpea). To determine short-term effects, subsets of individuals from each population were exposed to the alternative host for 1 generation. We revealed higher level of indiscriminate mating in populations, which evolved on the suboptimal host. Males from these populations spent less time in assessing mates although they had the ability to discriminate between signals. Short-term larval experience on the suboptimal host also decreased selectivity of mates. The results imply that plastically induced reduction in mate discrimination, after a shift to suboptimal host, may have been canalized through long-term genetic changes underlying behavioral development. Significant reproductive isolation between the 2 sets of populations was revealed regardless of the short-term host treatment.

Key words: **host-shift, mating behavior, phytophagous insects, sexual isolation.**

INTRODUCTION

To understand the evolution of mate choice, it is important to comprehend 2 major components of courtship interactions: 1) mating preference that presumes the propensity of females and males to mate with certain phenotypes (Jennions and Petrie 1997; Cotton et al. 2006) and 2) mate signaling, that is, the expression of sex-specific phenotypes on which mating decisions are made, and which cover a range of visual, auditory, vibrational, and chemical modalities (e.g., review in Coyne and Orr 2004; Nosil 2012). As elaborated by Jennions and Petrie (1997) and Cotton et al. (2006), mate choice is the active manifestation of mating preference, and, generally, it can evolve if preferences of 1 sex coevolve with signals of the other. In order to improve theoretical and empirical treatment of these phenomena, Jennions and Petrie (1997) further subdivide preference into 2 properties of individuals: 1) preference function—a ranked order of prospective mates with respect to traits relevant for sexual interactions and 2) choosiness—the effort an individual invests in assessment of mates. Variation in choice of sexual partners could be, therefore, due to variability in preference function, choosiness, or both. At the more proximate level, mating preferences, and consequently mate choice, largely depend on the ability of individuals to perceive signals and/or to discriminate between stimuli (Jennions and Petrie 1997; Ronald et al. 2012).

It has long been recognized that environmental variation can influence mating patterns in 1 population through effects on development of mating signals and preferences (David et al. 2000; Hunt et al. 2005; Cotton et al. 2006; Smadja and Butlin 2009; Eraly et al. 2009; Cocroft et al. 2010; Geiselhardt et al. 2012). Studies concerned with environmental influences on sexual behavior have formulated "developmental stress hypothesis" (Nowicki et al. 2002; Buchanan et al. 2003), which proposes that disturbing effects of novel conditions on developing sensory systems can consequently change perception of signals in mate courtships. At the same time, altered environments may induce plastic changes in expression of mating signals (e.g., Qvarnström et al. 2000; Nosil et al. 2007; Rodríguez et al. 2008; Etges et al. 2009; Cocroft et al. 2010). In insects, great number of studies analyzed modifications in CHCs (Howard and Blomquist 2005), which function as contact pheromones in their mating systems (Tregenza et al. 2000; Ferveur 2005).

Address correspondence to B. Stojković. E-mail: bilja@bio.bg.ac.rs.

© The Author 2014. Published by Oxford University Press on behalf of the International Society for Behavioral Ecology. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

It was found that variation in these compounds reflected both genetic variability (Havens and Etges 2013 and references therein) and environmental influences on CHC biosynthesis (e.g., Grace and Shaw 2004; Etges et al. 2007; Cocroft et al. 2008; Fedina et al. 2012) due to changed "molecular architecture that underlies plasticity of gene expression" (Smith et al. 2013, p. 1961).

Novel phenotypic variability induced by environmental changes, if associated with altered reproductive fitness, can trigger new courses of mating pattern evolution. As elaborated by Fitzpatrick (2012), these short-term plastic effects have influence on long-term evolutionary changes in a population if environmental impacts on genotypes and patterns of selection exhibit transgenerational similarity, that is, if natural and/or sexual selection consistently favor particular developmental pathways. Regarding evolutionary changes of phenotypic plasticity per se, either increase or decrease in environmental sensitivity of traits can be expected (Crispo 2007; Pfennig et al. 2010). One possible outcome of this process is the genetic assimilation wherein induced phenotype loses its environmental sensitivity and becomes a constitutively expressed trait through quantitative genetic change (Waddington 1953; West-Eberhard 2003; Suzuki and Nijhout 2006; Badyaev 2009). Following this scenario, divergent mating cues and preferences, which can be expected between individuals developed in different habitats, may instantly reduce probability of interpopulation mating (Cocroft et al. 2010; Matsubayashi et al. 2010; Geiselhardt et al. 2012), whereas plastically induced changes in genetic variation in each population and divergent selection regimes acting on associated behavioral traits can lead to the long-term evolution of premating reproductive isolation (RI) between populations (Pfennig et al. 2010; Fitzpatrick 2012).

In this study, we investigate the short- (plastic) and long-term (evolutionary) effects of the host-shift on mating behavior and levels of pre-mating RI in the seed-eating beetle *Acanthoscelides obtectus*. The experiments were performed on laboratory populations that were raised for 50 generations (approximately 4 years) on the suboptimal host seed (chickpea) and populations maintained on the optimal host (common bean). Using populations from these 2 selection regimes, we had the opportunity to investigate long-term evolutionary changes in mating system after the host-shift event. In addition, by rearing beetles for 1 generation on alternative hosts in reciprocal transplant experiments, we analyzed initial plastic influence of novel host on mating behavior (i.e., shift from been to chickpea) and changes in plasticity of behavioral traits after 50 generations of evolution on suboptimal host (i.e., shift from chickpea to bean). By performing crosses between beetles originated from the same or different selection regimes and short-term rearing hosts, we evaluated the levels of RI among populations caught in different phases of the host-shift. In searching for the causes of long- and short-term changes in mate choice, we analyzed variation in the 2 components of mating preference in interpopulation crosses. Preference function for each type of female and male, regarding their origin and rearing host, was obtained by plotting their copulation frequencies in relation to all different sources of mates, that is, mates that originated from the same or alternative long- and short-term hosts. For choosiness, we presumed that the elapsed time to initiation of copulation could be used as an approximation of the effort an individual invested in gathering information about potential mates (see e.g., Sullivan 1994; Ronald et al. 2012). Similarly to the preference function, choosiness was evaluated for each female and male type across all different sources of mates. Furthermore, because our previous findings on chemical signaling demonstrated both plastic and long-term changes of CHC profiles in *A. obtectus* populations (Savković et al. 2012), we correlated mating frequencies with quantitative values of CHC compounds in all combinations of crosses in order to find a link between mate choice and CHC signaling. Using all these data, we tested 2 major hypotheses. First, we tested whether plastic short-term changes of mating preference, in response to host-shift, could affect levels of behavioral isolation between laboratory populations of *A. obtectus*. Second, if the short-term rearing host effects on sexual behavior can indeed induce long-term divergent evolution of mating system in populations inhabiting alternative hosts, then 2 outcomes could be expected: 1) the patterns of long-term changes in behavioral traits should resemble those environmentally induced (Badyaev 2005) and 2) levels of assortative mating, that is, sexual isolation, should be higher among populations evolved for a long time on different hosts compared with levels of plastically induced isolation.

MATERIALS AND METHODS

Study species

The seed-eating beetle, *A. obtectus* (Say; Coleoptera: Chrysomelidae: Bruchinae) is a cosmopolitan pest of stored legumes. Although the common bean (*Phaseolus vulgaris* L.) is its primary plant host, this beetle is also capable to complete development on other, suboptimal, plant species within Fabaceae family (see Savković et al. 2012). Larval development and pupation complete entirely within a dry seed, and after approximately 30 days, adults emerge. Adults are facultative aphagous and are able to mate and complete their life cycles using only metabolic water and resources acquired during larval development. The pattern of courtship activities in this species does not include specific rituals or acoustic signals. Males chase and then mount females in orientation typical for insect copulation. Interestingly, the same behaviors have been observed in homosexual interactions in both sexes (Stojković et al. 2010). Although aggressiveness of males was perceived as the most important determinant in mating, it was shown that females also had active roles in mate discrimination leading to the pre-mating isolation between some laboratory populations (Stojković et al. 2011).

Laboratory populations

All populations used in this study originated from 1 "synthetic" population established from 3 storages in the vicinity of Belgrade (Serbia) 27 years ago (in 1986) and ever since this population was maintained on bean seeds in our laboratory. Four years ago, large samples of beetles, approximately 1000 adults per sample, were used to set up 8 replicate populations, 4 of which were reared on the optimal host, that is, bean (hereafter referred to as the "*Phaseolus*" or P populations), and the other 4 were maintained on chickpea, *Cicer arietinum* L. (hereafter referred to as the "*Cicer*" or C populations). These populations evolved for 50 generations on alterative hosts prior to the experiment described in this study.

Beetles were reared in a dark incubator at about 30 °C and 70% humidity. All seeds were brought from 1 source and were frozen before use in the experiments. No food or water was offered to experimental adults.

Experimental design

The experiment was designed to simulate several phases of the host-shift in *A. obtectus* (Figure 1A). The first set of populations was represented with 4 P replicate populations, that is, populations

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

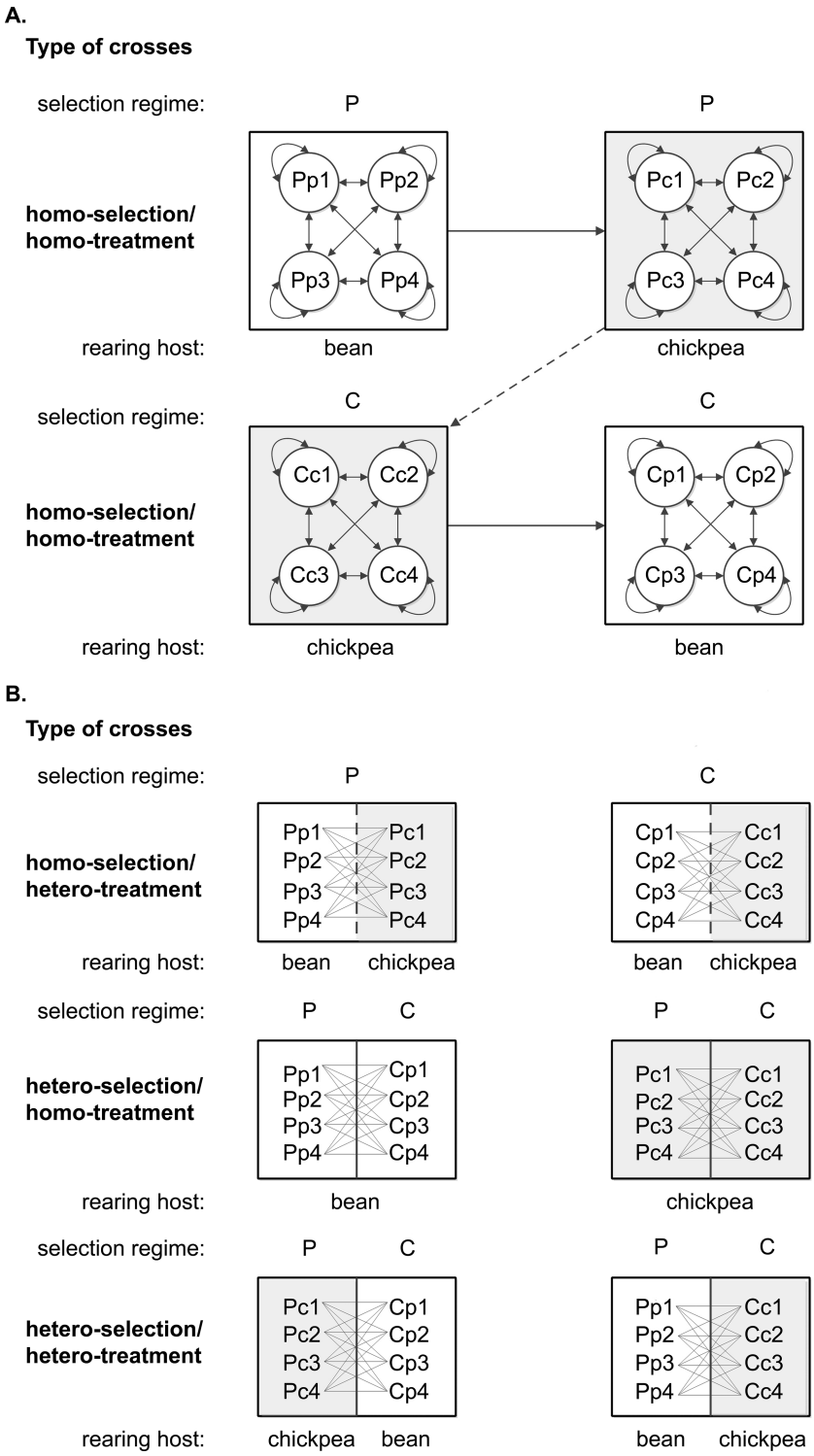

Figure 1

Experimental design and mating trials. (A) 4 experimental groups, each with 4 replicate populations. Pp group refers to populations adapted to beans, whereas Pc points to beetles originated from Pp populations but developed for 1 generation on chickpeas. Cc populations have been reared on chickpeas for 50 generations, and Cp group originated from these populations but developed on beans for 1 generation. Following the sequence of events in the host-shift process, Pc treatment could be presumed as the first step that preceded long-term evolution on a novel host (dashed arrow on the figure). Within each group, crosses within and between replicate populations were performed. This type of crosses was termed homo-selection/homo-treatment. (B) Types of crosses between experimental groups. In homo-selection/hetero-treatment, cross type individuals from the P and C selection regime were paired to beetles originated from the same regime but developed on the alternative host (Pp × Pc and Cc × Cp). Hetero-selection/homo-treatment mating category was presented with crosses between beetles originated from different selection regimes but developed on the same plant host (Pp × Cp and Pc × Cc). In hetero-selection/hetero-treatment mating type, both the origin and rearing hosts were different between interacting individuals (Pc × Cp and Pp × Cc). Within each mating category, all combinations of crosses between 4 replicate populations were performed. All matings were designed as reciprocal crosses (solid lines). Hosts on which beetles were developed are represented with different colors of panels—white for bean and grey for chickpea.

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

that were well adapted to the optimal host. Being that P selected experimental beetles completed their larval development on bean, we denoted this experimental group as Pp group. In the next step, we assumed that the environment of these populations became heterogeneous by introducing seeds of a novel host plant, that is, chickpea. This scenario is highly probable for this species because it was argued that mixing of legume species in storages enabled *A. obtectus* to expand the range of acceptable hosts following trade routes all over the world (Alvarez et al. 2005). Further, depletion of the preferable host forced mated females to lay eggs on less suitable host and a new generation developed on chickpeas. To determine these short-term developmental effects of the host-shift on behavioral traits, we created experimental group in which chickpea was offered to mated P individuals; they deposited eggs and their larvae completed development on a novel host. The adults emerged from these seeds were referred to as Pc experimental group. In the next step, we assumed that the optimal host was no longer available and populations were allowed to evolve on chickpea. These long-term host-shift effects were analyzed using populations reared for 50 generations on chickpeas (i.e., C populations). In the experiment, we used beetles that were also developed on chickpea and, therefore, we termed this experimental group as Cc group. Finally, populations adapted to a novel host might perform a secondary contact with beans, exposing eggs and larvae to the previously optimal host species. Experimental group, named Cp, which was created to determine this secondary short-term developmental effect on mating behavior, consisted of individuals that originated from C populations but completed their larval development on beans. In sum, 4 experimental groups were assayed in this study, each of which had 4 replicate populations resulting in 16 population/rearing host combinations (i.e., 4 Pp and Cc, and 4 Pc and Cp replicates; Figure 1A).

Mating trials

Mating trials were no-choice in design, as suggested by Rolán-Alvarez and Caballero (2000) because this procedure allowed monitoring of precise numbers of potential and realized copulations in each mating combination (Figure 1). This method enables assessment of sexual isolation parameter (I_{PSI}) that is based mainly on mate choice and decreases the effects of among-population variations in mating propensities. In addition, all matings were designed as reciprocal crosses meaning that each combination of populations was represented with a pair of crosses that reversed the sexes associated with each population (see Carvajal-Rodrigues and Rolán-Alvarez 2006). It must be kept in mind, however, that although this design enabled precise assessment of mate choice isolation, it precluded any conclusion on the roles of intrasexual competition in beetles' mating decisions. To evaluate the relevance of competitive interactions in behavioral isolation between these populations, further analyses are needed.

All 16 population/rearing host cultures were inspected several times per day and newly emerged virgin females and males were collected within 2 h. Sexes were separated in different bottles to prevent copulation prior to mating trials and were kept under constant conditions at about 30 °C and 70% humidity in a dark incubator. Experimental beetles were 1 day old. Single female/male pairs were placed into observation chambers (30-mm Petri dishes without beans) and monitored for 30 min. For each pair of virgin beetles, we recorded 2 measures of reproductive behavior: the successful copulation (yes or no) and, if copulation occurred, copulation latency (the elapsed time to initiation of copulation). Mating behavior was evaluated for all population/rearing host combinations, both within and between experimental groups (Figure 1).

This complex design of cross combinations can be organized in 4 hierarchical levels (Figure 1), where each level reflects different aspects of long- and short-term host effects on sexual behavior. The first group of crosses, denoted as "homo-selection/homo-treatment," evaluated levels of assortative mating among populations of the same origin (i.e., the same selection regime) and the same nutritional larval experience. These crosses were needed for all calculations of RI parameters and behavioral comparisons because they served as a standard for the observed/expected mating ratio. In the second group of crosses, labeled as "homo-selection/hetero-treatment," mating partners originated from the same selection regime but were developed on alternative host seeds. These crosses explained the short-term plastic change in sexual behavior and its influence on mating patterns after the initial exposure of larvae to novel hosts for 1 generation. The third group of crosses, called "hetero-selection/homo-treatment," consisted of female/male pairs that originated from populations adapted to diverse hosts, but these adults experienced seeds of the same plant species during their larval development. The goal of this cross type was to assess the levels of partners' recognition and mate choice in situations where beetles, which evolved under divergent selection pressures, were found in the same environment. In each of the mating combinations within this cross type, the developing environment was common for 1 mating partner and novel for the other. In the fourth type of crosses, both the origin of experimental populations and larval experience were different, so this group was named "hetero-selection/hetero-treatment." Within this cross type, 2 questions could be asked: 1) How large is the influence of plastic developmental changes in sexual behavior on patterns of mate choice when both partners were exposed to novel hosts during their larval development (Pc × Cp crosses)? and 2) If populations evolved for a long time on alternative hosts and their adults developed on their common host species, what is the level of pre-mating RI between these populations (Pp × Cc crosses)? The last question points specifically to the long-term evolutionary effects of host-shift on sexual isolation. In all other cross types, we investigate the influence of rearing host on expression of behavioral traits and its consequence on the RI between populations with different evolutionary and developmental histories. Within each cross type, we performed crosses in all combinations of populations (Figure 1). There were 2 reasons for this multiplication of crosses. First, stochastic variation between populations within selection regimes, due to genetic drift, was ruled out in that way and detectable short- and long-term host effects on experimental groups could be confidently attributed to the outcomes of selection. Second, by controlling identity of population for both females and males, we obtained 64 mating frequency data for each cross type (32 for females and 32 for males), which improved statistical analyses of these records.

Each cross combination among populations was represented with 40 female/male pairs for direct and 40 pairs for reciprocal crosses. As mating experiments lasted for 15 days, we standardized the procedure in order to decrease the effect of environmental fluctuations on behavioral variation. First, all trials were carried out in the morning, between 9:00 and 13:00 h. Second, the temperature in the laboratory was kept constant at 26 °C. Third, within each cross combination, mating trials were randomized between at least 5 days. Using this procedure, a potential day-to-day variation was included into the total behavioral variance for each cross type.

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

CHCs assays

Results on chemical identity and quantity of detected CHCs were taken from our previous study on the same populations of *A. obtectus* (Savković et al. 2012), where each of 32 experimental groups (2 selection regimes × 2 developing hosts × 4 populations × 2 sexes) were represented with 31–50 virgin individuals. Individuals within each sample were rinsed together with 2 mL of redistilled *n*-hexane followed by 5 s of vortex sessions before and after 10 s in an ultrasonic bath (50/60 Hz). Prepared samples with rinsed CHC compounds were then transferred into chromatography vials (Agilent Technologies, Screw caps vials, 1.5 mL) for the gas chromatography-mass spectrometry (GC-MS) analysis. The GC and GC-MS analyses were performed on an Agilent 7890A GC system equipped with a 5975C inert XL EI/CI MSD and a FID detector connected by capillary flow technology through a 2-way splitter with make-up gas. An HP-5 MSI capillary column (Agilent Technologies, 25 mm inner diameter, 30 m length, 0.25 μm film thicknesses) was used. Each sample had injection volume of 1 mL. Carrier gas flow rate and column temperature sequence are described in Savković et al. (2012).

Database search, mass spectral deconvolution, and extraction were achieved using NIST Automated Mass Spectral Deconvolution and Identification System software, version 2.64 (NIST AMDIS 2005). Based on the retention times of *n*-alkanes that were injected after the sample under the same chromatographic conditions, retention index calibration data analysis was conducted. Characteristic spectral fragmentation patterns of normal, internally branched mono-, di- and tri-methylalkanes of electron impact mass spectra (Blomquist et al. 1987; Blomquist and Bagnéres 2010), combined with their retention indices (Carlson et al. 1998), led to the identification of CHC components, and their abundances were computed from the corresponding GC-flame ionization detector peak areas.

Data analyses and graphical presentation

Preference function

Copulation frequency, that is, the ratio between the number of successful copulations and the total number of pairs, was obtained for direct and reciprocal crosses in each pair of populations both within and between 4 experimental groups (Figure 1). In order to visualize preference function for each female or male type (i.e., Pp, Pc, Cc, and Cp), we plotted their mean copulation frequencies in relation to all different sources of mates. Frequency data on cross combinations were pooled within experimental groups. For example, to obtain mean copulation frequency of Pp females with Cc males, all combinations of population crosses within these 2 experimental groups were pooled—that is, $Pp_1 \times Cc_1$, $Pp_1 \times Cc_2$, $Pp_1 \times Cc_3$, $Pp_1 \times Cc_4$, $Pp_2 \times Cc_1$, $Pp_2 \times Cc_2$, … , $Pp_3 \times Cc_1$, … , $Pp_4 \times Cc_1$, … $Pp_4 \times Cc_4$ (16 population cross combinations; the numbers denote replicate populations). The same procedure was used to assess mean copulation frequency of Pp females with Pp, Pc, and Cp males. As a result, preference function curve consisted of 4 data points representing mean copulation frequency in crosses Pp × Cc, Pp × Pc, Pp × Cc, and Pp × Cp. This method was employed for each female and male type. In order to test overall changes in preference function within each female and male type, copulation frequencies across their mate types were compared using G-test of homogeneity (Sokal and Rohlf 1995). In addition, mating success was compared between each 4 points in preference function curve using G-test. The obtained G-values were compared with the critical value of χ^2 for (n – 1) degrees of freedom.

Choosiness

Similarly to the preference function, for each female and male type, choosiness was graphically presented using data on copulation latency in sexual interactions with all types of mating partners, that is, 4 data points represented changes in choosiness when certain type of individuals was faced with different types of mates. Again, population origin of individuals was pooled within experimental groups, and mean values and their errors were used for graphical presentation and statistical comparisons. To test for differences in mean pre-copulation time between 4 data points within each female and male type, Mann–Whitney *U* nonparametric test was used. Mixed-effect nested Anova (SAS Institute, Inc 2010) was performed to analyze the effects of selection regime (long-term host), rearing host (short-term effect), sex, and population origin on the observed variance of copulation latency. Replicate populations were designated as random factors and were nested within selection regime × rearing host interaction because our experimental groups were defined by both factors. Anova was performed on log-transformed data on copulation latency to improve normality.

Pre-mating isolation

The magnitude of sexual isolation between populations was estimated using the I_{PSI} index (0 = random mating, +1 = complete sexual isolation), which could distinguish among real mate choice and differential mating propensity. This parameter evaluates a deviation from random mating in mated individuals (i.e., assortative or disassortative mating) and is calculated by comparing the number of copulating pairs and expected mating frequencies in each cross combination (Rolán-Alvarez and Caballero 2000). Using JMating software, version 1.0.8 (Carvajal-Rodrigues and Rolán-Alvarez 2006), we also examined the significance of mating propensity coefficients (*PSS*, indicates general willingness of individuals to be involved in copulation) by G-test, and the cross-product estimator (*W*) that is considered as the maximum likelihood reproductive fitness estimator of 1 morph type relative to the other (e.g., female Pp relative to Pc female in Pp × Pc crosses). Significance of all JMating statistics was determined by resampling 10 000 times with JMating software, version 1.0.8 (Carvajal-Rodrigues and Rolán-Alvarez 2006).

CHC signaling and copulation frequency

To investigate relationship between mating signaling and preference, we estimated correlations between quantitative measures of hydrocarbon compounds and copulation frequencies in each cross combination. Female signals were correlated with mating frequencies regarding male type in each cross combination, and the same procedure was applied for male signals and female mating frequencies. Because chemical assays were performed on a group level (i.e., not individually; see earlier), multivariate methods had low statistical power and were not performed on CHC data. Instead, correlations with copulation frequency were assessed for each compound after centered logratio (clr) transformation of CHC records (Aitchison 1986; Pawlowsky-Glahn and Buccianti 2011) using CoDaPack software (Comas-Cufí and Thió-Henestrosa 2011). Proportion data (copulation frequency) were arcsine(sqrt) transformed to improve normality. Pearson correlation coefficients were calculated using PROC CORR procedure in SAS software (SAS Institute, Inc 2010). Bonferroni correction for multiple comparisons of 21 CHC compounds was applied for each mating type (Rice 1989).

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

RESULTS

Preference function

Experimental groups of *A. obtectus*, both females and males, showed differences in patterns of preference function. Changes within preference function were significant for Pp, Cc, and Cp females, and for males in Pp and Pc experimental groups, whereas for Pc females and Cc and Cp males, copulation frequencies did not change significantly across mate types (Table 1). The shapes of preference functions for each female and male type are presented in Figure 2. Compared with the interactions with males from their own experimental group, copulation frequency of Pp females significantly dropped and remained mostly constant when faced with males from all other experimental groups (Figures 2A). Similarly, significant decrease in copulation frequency was observed in Pp males when interacting with other experimental groups with the lowest frequencies in sexual interactions with C females (both Cc and Cp; Figure 2B). Development on chickpea for 1 generation resulted in a flat preference function of Pc females, that is, copulation frequencies were similar regardless of mate type (Figure 2A). General decrease in mating frequency of Pc females compared with the original Pp populations was observed in comparison between intra-group crosses (Pp × Pp vs. Pc × Pc: $G_{(1)} = 11.36$, $P < 0.001$). Preference function of Pc males paralleled the function of Pc females for all mate types except for Cp females where copulation frequency significantly increased leading to the heterogeneous preference function (Table 1; Figure 2B).

In C selection regime, the short-term development on alternative host species (i.e., beans; Cp experimental group), in comparison with Cc beetles, significantly increased proportion of successfully copulated pairs in all combinations (overall G-value across all mate types for females $G_{(1)} = 9.52$, $P < 0.01$ and $G_{(1)} = 7.11$, $P < 0.001$ for males). The lowest mating frequencies for Cc and Cp females were observed in interactions with Pp males and increased toward the highest values in sexual contacts with Cp males (Figure 2C). This gradual increase across mate types resulted in heterogeneous preference functions for Cc and Cp females (Table 1). On the other hand, although copulation frequencies for Cc and Cp males were also the highest when interacting with Cp females, there were no significant differences across mate types (Figure 2D), and overall preference functions were homogenous (Table 1).

Choosiness

The analyses of copulation latency showed significant long- and short-term effects on the time that different types of individuals spent in pre-copulation period of sexual interactions (Table 2). We found no significant differences between replicate populations indicating that the role of genetic drift in population divergence, regarding this component of reproductive behavior, was not profound. On the other hand, the long-term divergent selection pressures on alternative host species resulted in significantly faster copulation, that is, shorter time spent in assessment of mates, in C compared with P selection regime ($F = 13.84$, $P < 0.0027$; Table 2). Although rearing host did not have significant effect on total variance of copulation latency, the importance of the short-term effect on sexual behavior was determined by its interaction with selection regime indicating different patterns of plastic responses between the 2 long-term regimes ($F = 36.73$, $P < 0.0001$; Table 2). We also found no differences between the sexes in copulation latency, but significant sex × selection regime interaction term implied that females and males differed between selection regimes ($F = 11.73$, $P < 0.0006$; Table 2).

Changes in copulation latency across mate types for each type of females and males are presented in Figure 3. Among all crosses, the longest period of time before copulation was found in Pp × Pp combinations. These beetles decreased the time spent in mate assessment when they were faced with other mate types with no significant differences between them. After 1 generation of development on suboptimal host, compared with Pp beetles, Pc individuals decreased their assessment time when interacting with all mate types (Pp = 10.81 ± 0.67 min, Pc = 7.71 ± 0.20 min; Mann–Whitney's $U_{(4,4)} = 0$, $P < 0.05$). The fastest copulation achievements of Pc individuals were revealed when they interacted with Cp beetles.

In C selection regime, Cc and Cp individuals of both sexes generally delayed copulation more when interacting with P beetles than in interactions with C beetles (Figure 3). Short-term larval experience on beans resulted in lower copulation latency in Cp males compared with Cc males although this difference was not significant (Cc = 7.86 ± 0.40 min, Cp = 6.75 ± 0.49 min; Mann–Whitney's $U_{(4,4)} = 0$, $P < 0.34$) because of the increase in assessment time in Cp × Cp crosses.

Regarding the host-shift scenario that we described earlier (see Experimental design for details), we found that both short- and long-term shifts influenced sexual behavior. When compared with populations well adapted to the optimal host plant (Pp populations), overall copulation frequency across all mate types after developing on a novel host (Pc beetles) significantly decreased (pooled sexes, Pp vs. Pc: $G_{(1)} = 16.63$, $P < 0.001$). Similar trend was observed in comparisons between Pp populations and effects of long-term evolution on chickpea (pooled sexes, Pp vs. Cc: $G_{(1)} = 2.17$, $P < 0.001$; Pp vs. Cp: $G_{(1)} = 10.63$, $P < 0.01$). As noted earlier, choosiness in mate interactions was decreased after the short-term development on a novel host, as well as after the long-term evolution on chickpea (pooled sexes: Pp = 10.81 ± 0.67 min, C [Cc, Cp] = 7.63 ± 0.14 min; $U_{(4,8)} = 0$, $P < 0.05$).

Table 1

G-test of heterogeneity in preference functions for females and males within each experimental group across all mating types

Preference function		G_{Total}	G_{Pooled}	$G_{H\,(df)}$	*P*
Females	Pp	42.88	19.44	23.44 $_{(5)}$	0.0003
	Pc	4.69	4.05	0.65 $_{(5)}$	0.9856
	Cc	22.24	9.60	12.64 $_{(5)}$	0.027
	Cp	26.64	13.28	13.36 $_{(5)}$	0.0203
Males	Pp	63.69	42.23	21.46 $_{(5)}$	0.0007
	Pc	18.23	0.60	17.63 $_{(5)}$	0.0035
	Cc	11.74	3.46	8.29 $_{(5)}$	0.1412
	Cp	6.03	1.09	4.94 $_{(5)}$	0.4227

Pre-mating isolation between experimental groups

Mating propensities and reproductive fitness indices were the highest for the Pp individuals in all combinations of crosses (Table 3). After the first step of host-shift to chickpea (Pc group), the willingness of beetles to be involved in sexual interactions significantly dropped. Still, this decrease in mating propensity did not result in pre-mating RI between original populations and individuals developed on alternative host (Pp × Pc crosses:

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

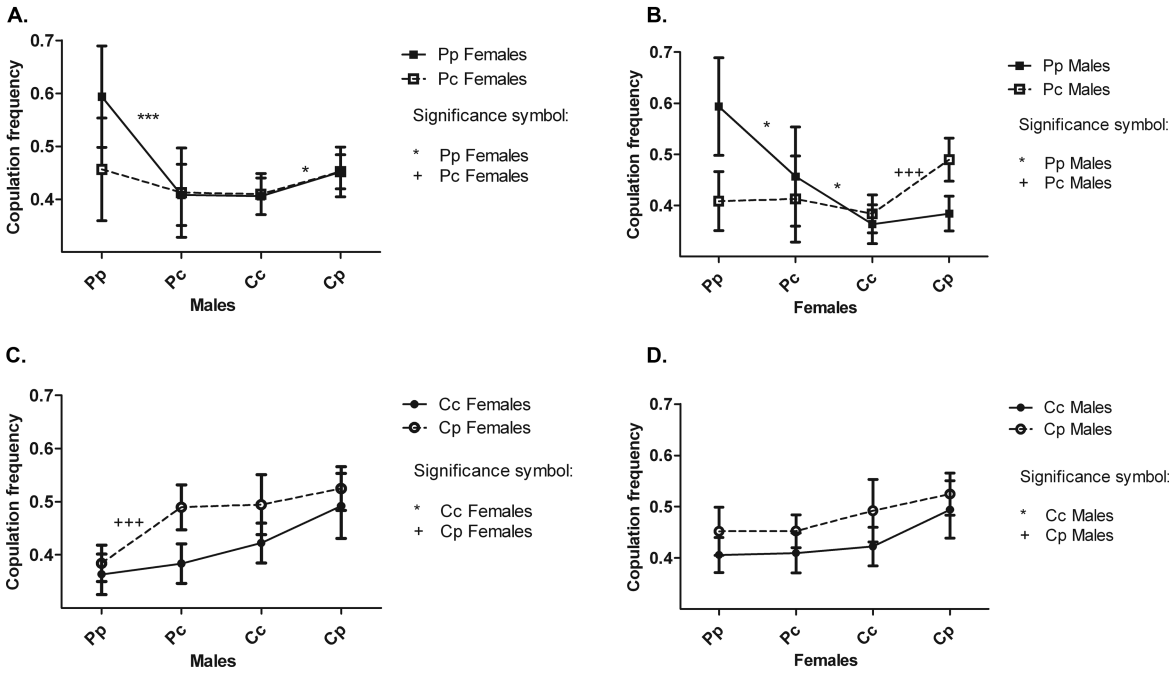

Figure 2

Preference function obtained by plotting copulation frequency for each female type ([A] Pp and Pc, [C] Cc and Cp females) and each male type ([B] Pp and Pc, [D] Cc and Cp males) in relation to different sources of mates. Means (± standard error) are presented. For each type of individuals, differences between mating combinations were determined by G-test. Significant differences are indicated by * and + symbols; ***, $^{+++}P < 0.001$, *, $^{+}P < 0.05$.

Table 2

Mixed-effect nested Anova for copulation latency (choosiness)

	Choosiness		
	df	*F*	*P*
Selection regime	1	13.84	0.0027
Rearing host	1	1.02	0.3325
Sex	1	0.01	0.9254
Selection regime × rearing host	1	36.73	0.0001
Sex × selection regime	1	11.73	0.0006
Sex × rearing host	1	0.88	0.3469
Population (selection regime × rearing host)	12	0.63	0.8216

Selection regime, rearing host, and sex were fixed factors, and replicate population, nested within selection regime × rearing host interaction, was random factor in Anova. df, *F*, and *P* values are presented.

$I_{PSI} = 0.09$, $P < 0.1$; Table 3). Similarly, within the other group of long-term regimes, that is, Cc and Cp beetles, sexual isolation did not emerge (Cc × Cp crosses: $I_{PSI} = 0.02$, $P < 0.8$; Table 3). However, the long-term adaptations to alternative hosts undoubtedly resulted in significant pre-mating RI between P and C populations regardless of the short-term host influence in C populations (Pp × Cc crosses: $I_{PSI} = 0.14$, $P < 0.0001$; Pp × Cp crosses; $I_{PSI} = 0.12$, $P < 0.0001$).

Interestingly, the short-term changes in sexual vigor in C and P populations showed the opposite trends. Although mating propensity and reproductive fitness decreased in P populations after developing on chickpea (Pc group), bean seeds induced increase in these reproductive traits in C populations (Cp group). Although this elevation in Cp group was not significant when compared with C populations (Cc × Cp crosses), it was revealed as significantly higher in comparison with Pc individuals (Pc × Cp crosses: G-test for mating propensity, $G_{(1)} = 6.46$, $P < 0.046$; $W_{(Pc)} = 0.86$, $W_{(Cp)} = 1$, $P < 0.009$; Table 3). These changes in reproductive behavior resulted in the loss of RI between Pc and Cp groups ($I_{PSI} = 0.02$, $P < 0.6$), as well as among Pc and Cc populations ($I_{PSI} = 0.04$, $P < 0.3$; Table 3).

CHC signaling and mating preference

GC and GC-MS analyses identified 21 different compounds of CHCs in *A. obtectus*. The complete list of CHCs and their abundances can be found in Savković et al. (2012). Analyses of correlation pattern between CHCs quantities and copulation frequencies within each cross type did not reveal any significant correlation coefficient in homo-selection cross combinations. Also, we did not find any significant relationship between female preference functions and male CHC signatures after Bonferroni corrections (critical $P < 0.002$). Thus, only correlation results on hetero-selection crosses (i.e., Pp × Cc, Pp × Cp, Pc × Cc, and Pc × Cp) for male copulation frequencies and female CHC profiles were presented in Table 4. Only 4 female compounds were found significantly correlated with male copulation frequency and only for C males (Table 4). Males from P selection regime did not display any discrimination between potential mates with respect to mating frequency. Among various combinations of crosses, Cc and Cp males were able to discriminate only among females that evolved and developed on bean host (i.e., Pp females). When interacting with any other type of females, these males copulated randomly with regard to chemical signals. Three hydrocarbon compounds, *n*-pentacosane, 9-methylheptacosane, and *n*-hentriacontane, were significant for Cc males in discriminating between Pp females. Lower amount of *n*-nonacosane in Pp female cuticula was related to higher copulation frequency with Cp males (Table 4).

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

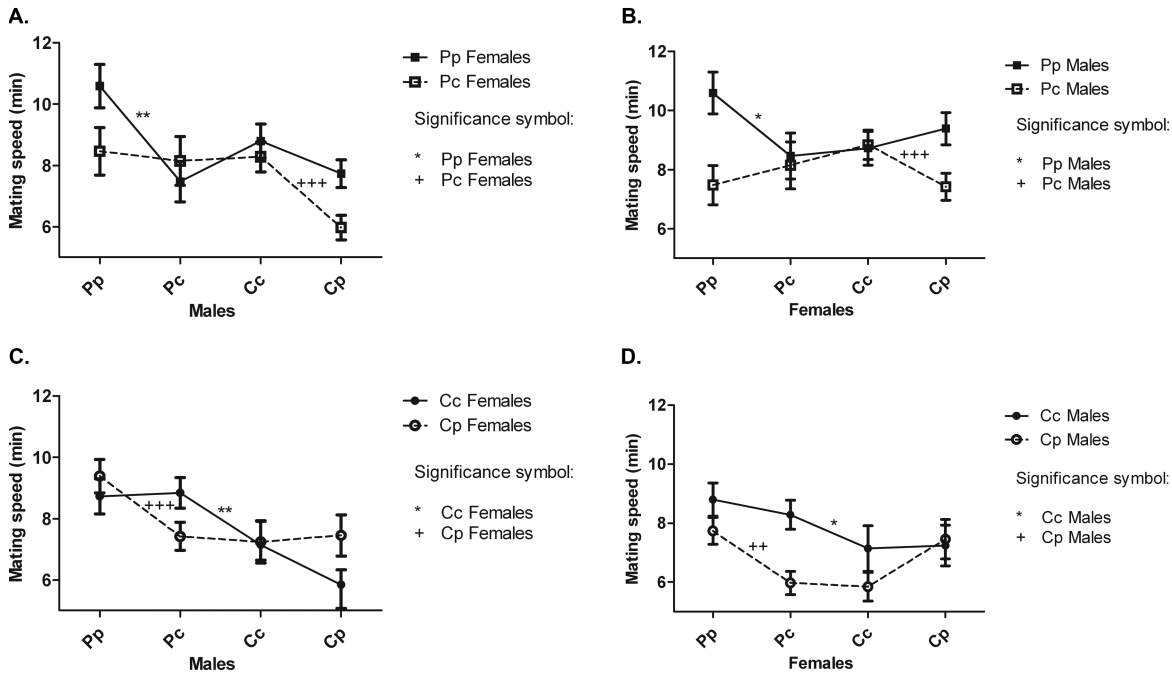

Figure 3

Choosiness function obtained by plotting time spent in sexual interaction prior to copulation for each female type ([A] Pp and Pc, [C] Cc and Cp females) and each male type ([B] Pp and Pc, [D] Cc and Cp males) in relation to different sources of mates. Means (± standard error) are presented. For each type of individuals, differences between mating combinations were determined by Mann–Whitney nonparametric test. Significant differences are indicated by * and $^{+}$ symbols; ***, $^{+++}P < 0.001$, **, $^{++}P < 0.005$, *, $^{+}$ $P < 0.05$.

Table 3

Sexual isolation indices (I_{PSI} parameter), G-values for mating propensity and relative reproductive fitness (W) for females and males in different mating combinations

Group	Cross combinations ♀ × ♂	Mating propensity G-test values probability	Reproductive fitness (W); bootstrap probability ♀	Reproductive fitness (W); bootstrap probability ♂	I_{PSI} values; bootstrap probability
Homo-selection/	Pp × Pc	**6.65**	Pp = 1	Pp = 1	0.09
hetero-treatment	Pc × Pp	**0.036**	Pc = 0.88; *0.093*	Pc = 0.80; **0.011**	0.103
	Cc × Cp	1.65	Cc = 0.91	Cc = 0.92	0.02
	Cp × Cc	0.440	Cp = 1; 0.167	Cp = 1; 0.194	0.761
Hetero-selection/	Pp × Cp	*5.58*	Pp = 1	Pp = 0.97	**0.12**
homo-treatment	Cp × Pp	*0.061*	Cp = 0.86; **0.011**	Cp = 1; 0.313	**0.000**
	Pc × Cc	0.59	Pc = 1	Pc = 0.95	0.04
	Cc × Pc	0.745	Cc = 0.99; 0.458	Cc = 1; 0.224	0.302
Hetero-selection/	Pc × Cp	**6.46**	Pc = 0.86	Pc = 0.94	0.02
hetero-treatment	Cp × Pc	**0.046**	Cp=1; **0.009**	Cp = 1; 0.162	0.576
	Pp × Cc	**11.97**	Pp = 1	Pp = 1	**0.14**
	Cc × Pp	**0.003**	Cc = 0.81; **0.002**	Cc = 0.89; **0.004**	**0.000**

Significance of all statistics was determined from 10 000 bootstrap resampling; bold type indicates differences for each pairwise combination significant at the $P < 0.05$, whereas italic type indicates marginally significant statistics at the $P < 0.1$.

DISCUSSION

First steps in host-shift and plasticity of mating behavior

Our study on *A. obtectus* demonstrated significant differences in reproductive behavior between beetles raised exclusively on optimal host (i.e., bean seeds) and those developed on chickpeas. In various insect taxa, it was shown that development of larvae on a novel host affected patterns of reproduction (Denno and McCloud 1985; Ernsting et al. 1992; Sokolovska et al. 2000; Awmack and Leather 2002; Boggs and Freeman 2005; Etges and Tripodi 2008; Eraly et al. 2009; Gosden and Chenoweth 2011; Lewis et al. 2012), as well as adult life-history traits (Messina and Fry 2003; Vanbergen et al. 2003; Huang et al. 2005; Messina et al. 2009) because these larvae have to cope with potential toxic substances or reduced nutritional intake (Awmack and Leather 2002 and reference therein). As noted by Cotton et al. (2006), under these circumstances, individuals may modulate their mating preferences because mating decisions depend on individual's ability to cover the cost or to benefit from discrimination between prospective mates, that is, variation in preference is condition-dependent.

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

Table 4

Correlations between male copulation frequency and female CHC amounts in different hetero-selection/hetero-treatment cross combinations

Compound name	Pp♀ × Cp♂	Cp♀ × Pp♂	Pp♀ × Cc♂	Cc♀ × Pp♂	Pc♀ × Cp♂	Cp♀ × Pc♂	Pc♀ × Cc♂	Cc♀ × Pc♂
Females choosed by males								
n-Pentacosane	ns	Ns	**0.717**	ns	ns	ns	ns	ns
9-Methylheptacosane	ns	Ns	**0.730**	ns	ns	ns	ns	ns
n-Nonacosane	**−0.724**	Ns	ns	ns	ns	ns	ns	ns
n-Hentriacontane	ns	Ns	**−0.747**	ns	ns	ns	ns	ns

Although 21 CHC compounds were detected in *Acanthoscelides obtectus* (Savković et al. 2012), we present only those compounds that are significantly correlated with copulation frequency in at least 1 cross combination. Significant correlation coefficients after Bonferroni correction are indicated as bold; ns refers to insignificant correlation, that is, $P > 0.002$.

Compared with all other types of crosses, when Pp beetles interacted with individuals from the same selection and rearing regime (Pp populations), all indices of reproductive success, that is, copulation frequency, mating propensity, and reproductive fitness, were the highest. In addition, because copulation latency also showed the highest values, it could be inferred that these beetles were able to devote much time to making final mate choice decision if potential mates came from the same set of populations. It might be hypothesized that evolution on optimal plant host resulted in highly synchronized signal emission/reception systems enabling beetles to resolve fine differences between potential mates and possibly to increase the probability of mating with a high-quality individuals (Castellano et al. 2012). However, when faced with beetles from other experimental groups, copulation frequency significantly decreased and Pp beetles mated randomly without preference toward any mate type. Discrimination between Pp and any other type of individuals indicated the plastic sexual behavior in Pp beetles. The reasons for this decrease in preference strength and choosiness of Pp beetles could be found in altered sexual signaling of prospective mates that were reared on the alternative host. Our previous study on *A. obtectus* revealed that identity of both long-term and short-term plant host significantly influenced CHC profiles in beetles (Savković et al. 2012). Similar studies on other insects clearly demonstrate that insect diet directly affects mating signals (e.g., Etges and Tripodi 2008; Geiselhardt et al. 2012; Havens and Etges 2013) and that nutritional quality can induce synthesis of classes of CHCs with opposing effects on mate attractiveness (some of them were attractive and other repulsive) sending conflicting sexual messages (Fedina et al. 2012). In other words, genotype-by-environment interactions in sexual displays can disrupt the information content and their reliability as signals of mate quality (Higginson and Reader 2009; Ingleby et al. 2013b).

The first step of the host-shift, that is, development of P beetles on chickpea for 1 generation (Pc group), was accompanied by significant decline in copulation frequency, mating propensity, and reproductive fitness compared with original Pp populations. Proportion of successful copulations and choosiness in Pc beetles were reduced even when they interacted with their own Pc group. Generally, developmental response of P beetles to the suboptimal host was accompanied by a loss of the ability to discriminate between prospective mates—these beetles became less plastic in their mating behavior. Although associated with reduced reproductive fitness in comparison to the Pp populations, it is likely that this mating strategy could maximize success in reproduction in situations when adequate quality evaluation of mates is not possible and mating assessment and preference have high reproductive costs (Hingle et al. 2001; Härdling and Kokko 2005; Hunt et al. 2005; Ingleby et al. 2013a).

Long-term host-shift effects on mating behavior

Our results indicate that long-term changes in mating preference paralleled, in a large degree, those plastic alterations that were induced by short-term host shift. As in Pc experimental group, both Cc and Cp beetles showed low levels of discrimination between potential mates and mean time spent in mate assessment was reduced compared with choosiness within Pp mating trials. After 50 generations of maintenance on a novel host, seed beetles displayed indiscriminative mating patterns. There are 2 possible explanations for the observed long-term changes in mating behavior. First, similarity between the short- and long-term effects may simply reflect consistent course of plastic response that is induced by chickpea in each subsequent generation with no significant transgenerational genetic changes that underlie development of behavioral traits. Comparisons between P and C beetles that were reared on the optimal host, however, ruled out this possibility. Namely, development in the same environment did not decrease the difference in mating preference function among Pp and Cp beetles. Second explanation for the long-term maintenance of indiscriminative mating strategy in C selection group suggests that the reduction in sensitivity toward mate identity could be genetically assimilated because it coincides with the phenotype initially produced by plastic short-term response. According to Badyaev (2005), if induced phenotypic (i.e., behavioral) variance can be channeled in same directions by existing organismal systems, and if these induced strategies are favored by selection (both during and after stressful event), they can subsequently be stabilized by selection and become assimilated in a population through quantitative genetic changes (Oyama 2000; West-Eberhard 2003; Suzuki and Nijhout 2006; Lande 2009; Pfennig et al. 2010; Duckworth 2013). As predicted by the genetic assimilation theory (Crispo 2007; Pfennig et al. 2010), we have observed a shift from plastic mating behavior in original Pp populations to nonplastic, indiscriminative mating strategy in C beetles. The similar pattern of assimilated plastic response was previously revealed in P and C populations regarding another behavioral trait—the oviposition behavior (Stojković et al. 2012). Namely, when P beetles were raised for 1 generation on chickpea, they increased the level of egg-dumping (i.e., laying of eggs in the absence of host seeds). This host-induced behavioral phenotype was maintained in C population for almost 200 generations, and additionally, became insensitive to larval rearing substrate. Cheng et al. (2008) proposed that general loss in environmental sensitivity

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

of behavioral traits might indicate increased potential of populations for host range expansion.

Another argument for the independent evolution of behavioral traits in C populations refers to plastic changes in mating propensity. Contrary to the P beetles which decreased their reproductive vigor after developing on chickpea, C beetles elevated the frequency of copulation and devoted less time in mate assessment when developed on beans. It could be hypothesized that these courses of behavioral plasticity evolution might be a significant adaptation to a novel host. Our analyses of various life-history traits on the same C laboratory populations indicated that these populations evolved and became well adapted to chickpea (manuscript in preparation). For example, high preadult viability on chickpea was significantly reduced after developing on beans for 1 generation (82% and 36% in Cc and Cp, respectively). Keeping this in mind, it could be suggested that host-induced aggressive and indiscriminate courtship interactions of Cp individuals represent a strategy in which resource allocation into reproductive efforts can increase a potential to overcome low larval viability and fecundity on beans. Additionally, our results implied that genetic stabilization of indiscriminate mating in C individuals evolved separately from the ability to perceive sexual signals. Ronald et al. (2012) proposed that reduction in mate choice might result from decreased ability to discriminate between mates or from reduced choosiness per se (i.e., individuals may neglect received signals). Interestingly, correlation patterns between copulation frequency and CHCs showed that indiscriminate C males were the only participants in sexual interactions that could rank prospective female mates regarding their chemical signatures. Although the short-term shift to suboptimal host induced loss of this ability (in Pc beetles), it seems that long-term evolution on chickpea regained this capacity in C males.

Mate choice isolation

Although the short-term effects on preference function and choosiness were substantial, these behavioral changes did not reach a threshold to significantly reduce interbreeding between beetles developed on alternative hosts for 1 generation (Pp × Pc and Cc × Cp crosses). However, the long-term modifications of reproductive behavior, which paralleled those environmentally induced, eventually did result in low but significant mate choice isolation between P and C populations. Behavioral patterns in all experimental groups imply that Pp individuals have the largest impact on mate choice. Their copulation frequency and choosiness significantly drop whenever they interacted with any other type of beetles. In other words, well-established signal emission/reception systems may have caused unwillingness of individuals from ancestral populations to interact with "strangers" who developed some other phenotypic characteristics, although these "strangers" might be unselective in mating decisions (such as Cc and Cp beetles).

It is unlikely that the observed behavioral isolation was purely environmentally caused for 2 reasons. First, we found no significant RI indices between Pp populations and Pc beetles that were exposed to the short-term host change. Contrary to our results, recent study on *Phaedon cochlearie* revealed significant sexual isolation (I_{PSI} parameter) between beetles feeding on different host plant species for only 42 days (Geiselhardt et al. 2012). Second argument for the hypothesis that P and C populations genetically diverged was significant mate choice isolation between Pp and C populations, regardless of rearing host of C beetles. Additionally, in searching for the effects of chemical signaling on reproductive success and mate choice, we have found significant correlations between CHC composition and copulation frequency only in crosses between Pp and C beetles (both Cc and Cp), that is, the long-term diverged populations. According to Etges and Tripodi (2008), who obtained similar results on *Drosophila mojavensis*, this could be expected if CHCs were more strongly involved in sexual isolation between populations than in intrapopulation mate choice, although individuals might show population-specific preferences for certain CHC profiles.

Following the host-shift scenario (i.e., Pp → Pc → Cc → Cp, see Experimental design for details), which was used as an approximation of the sequence of events that reflected evolutionary history of *A. obtectus* during its expansion, we obtained results that strongly supported the prediction that environmentally triggered phenotypic variability might be a critical early stage of divergent mate choice evolution after a host-shift. Original (Pp) populations exhibited high levels of assortative mating and plasticity of mating preference. The consequences of development on the suboptimal host for 1 generation (Pc group) were demonstrated through a loss of sensitivity toward identity of prospective mates. This reduction in mate discrimination did not disappear after 50 generations of selection and C populations exhibited the same behavioral pattern. Therefore, decreased plasticity of mating preference, observed on a long-term scale, might be an example of transgenerational canalization of development, which followed environmentally induced behavioral changes during a host-shift process (sensu, Waddington 1953).

Findings from this study are also interesting regarding the question whether phenotypic plasticity operates as a constraint to mate choice divergence or can promote this process (Fitzpatrick 2012). When Pp beetles, which likely control mate choice in interaction with other types of beetles, have been developed on a novel host (Pc group), short-term reduction in discrimination between mates had led to decreased levels of assortative mating and consequently to the loss of mate choice isolation between Pc and C populations. On one hand, our findings on the 2 long-term host regimes suggested that developmental plasticity might have triggered behavioral changes that ultimately resulted in certain levels of mate choice isolation. On the other hand, we demonstrated that plastic alterations in behavior of 1 dominant participant in sexual interactions (i.e., Pp beetles) could diminish mate choice isolation in a large degree. From these results, it is clear that the effects of plasticity on population divergence largely depend on the courses of evolution of plasticity per se within populations (Schlichting and Pigliucci 1998; Pigliucci 2001; Pfennig et al. 2010). Further studies on *A. obtectus* are needed to resolve the relationship between fitness traits related to individual quality, and cost and benefits from mating preferences.

FUNDING

The work was supported by Serbian Ministry of Education, Science and Technological Development (173007).

We are grateful to 2 anonymous reviewers and Associate Editor A. Maklakov for making numerous valuable comments on previous versions of the manuscript.

Handling editor: Alexei Maklakov

REFERENCES

Aitchison J. 1986. The statistical analysis of compositional data. London (UK): Chapman & Hall, Ltd.

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

Alvarez N, McKey D, Hossaert-McKey M, Born C, Mercier L, Benrey B. 2005. Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans. Mol Ecol. 14:1015–1024.

Awmack CS, Leather SR. 2002. Host plant quality and fecundity in herbivorous insects. Annu Rev Entomol. 47:817–844.

Badyaev AV. 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proc Biol Sci. 272:877–886.

Badyaev AV. 2009. Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. Philos Trans R Soc Lond B Biol Sci. 364:1125–1141.

Blomquist JG, Bagnéres AG. 2010. Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge (UK): Cambridge University Press.

Blomquist JG, Nelson DR, de Renobales M. 1987. Chemistry, biochemistry, and physiology of insect cuticular lipids. Arch Insect Biochem. 6:227–265.

Boggs CL, Freeman KD. 2005. Larval food limitation in butterflies: effects on adult resource allocation and fitness. Oecologia. 144:353–361.

Buchanan KL, Spencer KA, Goldsmith AR, Catchpole CK. 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). Proc Biol Sci. 270:1149–1156.

Carlson DA, Bernier UR, Sutton BC. 1998. Elution patterns from capillary GC for methyl-branched alkanes. J Chem Ecol. 24:1845–1865.

Carvajal-Rodriguez A, Rolan-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.

Castellano S, Cadeddu G, Cermelli P. 2012. Computational mate choice: theory and empirical evidence. Behav Processes. 90:261–277.

Cheng CW, Yang RL, Cheng IC, Horng SB. 2008. Egg-dumping: a strategy for host range expansion in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Ann Entomol Soc Am. 101:950–954.

Cocroft RB, Rodríguez, RL, Hunt RE. 2008. Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. In: Tilmon KJ, editor. Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. California (UK): University of California Press. p. 88–100.

Cocroft RB, Rodríguez RL, Hunt RE. 2010. Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. Biol J Linn Soc. 99:60–72.

Comas-Cufi M, Thió-Henestrosa S. 2011. CoDaPack 2.0: a stand-alone, multi-platform compositional software. Girona, Spain: Proceedings of the 4th International Workshop on Compositional Data Analysis.

Cotton S, Small J, Pomiankowski A. 2006. Sexual selection and condition-dependent mate preferences. Curr Biol. 16:R755–R765.

Coyne JA, Orr HA. 2004. Speciation. Sunderland (MA): Sinauer Associates.

Crispo E. 2007. The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. Evolution. 61:2469–2479.

David P, Bjorksten T, Fowler K, Pomiankowski A. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. Nature. 406:186–188.

Denno RF, McCloud E. 1985. Predicting fecundity from body size in the planthopper, *Prokelisia marginata* (Homoptera: Delphacidae). Environ Entomol. 14:846–849.

Duckworth RA. 2013. Epigenetic inheritance systems act as a bridge between ecological and evolutionary timescales. Behav Ecol. 24:327–328.

Eraly D, Hendrickx F, Lens L. 2009. Condition-dependent mate choice and its implications for population differentiation in the wolf spider *Pirata piraticus*. Behav Ecol. 20:856–863.

Ernsting G, Isaaks JA, Berg MP. 1992. Life cycle and food availability indices in *Notiophilus biguttatus* (Coleoptera, Carabidae). Ecol Entomol. 17:33–42.

Etges WJ, de Oliveira CC, Gragg E, Ortíz-Barrientos D, Noor MA, Ritchie MG. 2007. Genetics of incipient speciation in *Drosophila mojavensis*. I. Male courtship song, mating success, and genotype x environment interactions. Evolution. 61:1106–1119.

Etges WJ, de Oliveira CC, Ritchie MG, Noor MA. 2009. Genetics of incipient speciation in *Drosophila mojavensis*: II. Host plants and mating status influence cuticular hydrocarbon QTL expression and G x E interactions. Evolution. 63:1712–1730.

Etges WJ, Tripodi AD. 2008. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. VIII. Mating success mediated by epicuticular hydrocarbons within and between isolated populations. J Evol Biol. 21:1641–1652.

Fedina TY, Kuo TH, Dreisewerd K, Dierick HA, Yew JY, Pletcher SD. 2012. Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. PLoS One. 7:e49799.

Ferveur JF. 2005. Cuticular hydrocarbons: their evolution and roles in *Drosophila* pheromonal communication. Behav Genet. 35:279–295.

Fitzpatrick BM. 2012. Underappreciated consequences of phenotypic plasticity for ecological speciation. Int J Ecol. 2012:1–12.

Geiselhardt S, Otte T, Hilker M. 2012. Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. Ecol Lett. 15:971–977.

Gosden TP, Chenoweth SF. 2011. On the evolution of heightened condition dependence of male sexual displays. J Evol Biol. 24:685–692.

Grace JL, Shaw KL. 2004. Effects of developmental environment on signal-preference coupling in a Hawaiian cricket. Evolution. 58:1627–1633.

Härdling R, Kokko H. 2005. The evolution of prudent choice. Evol Ecol Res. 7:697–715.

Havens JA, Etges WJ. 2013. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. IX. Host plant and population specific epicuticular hydrocarbon expression influences mate choice and sexual selection. J Evol Biol. 26:562–576.

Higginson AD, Reader T. 2009. Environmental heterogeneity, genotype-by-environment interactions and the reliability of sexual traits as indicators of mate quality. Proc Biol Sci. 276:1153–1159.

Hingle A, Fowler K, Pomiankowski A. 2001. The effect of transient food stress on female mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni*. Proc Biol Sci. 268:1239–1244.

Howard RW, Blomquist GJ. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu Rev Entomol. 50:371–393.

Huang CC, Yang RL, Lee HJ, Horng SB. 2005. Beyond fecundity and longevity: trade-offs between reproduction and survival mediated by behavioural responses of the seed beetle, *Callosobruchus maculatus*. Physiol Entomol. 30:381–387.

Hunt J, Brooks R, Jennions MD. 2005. Female mate choice as a condition-dependent life-history trait. Am Nat. 166:79–92.

Ingleby FC, Hosken DJ, Flowers K, Hawkes MF, Lane SM, Rapkin J, Dworkin I, Hunt J. 2013a. Genotype-by-environment interactions for cuticular hydrocarbon expression in *Drosophila simulans*. J Evol Biol. 26:94–107.

Ingleby FC, Hunt J, Hosken DJ. 2013b. Heritability of male attractiveness persists despite evidence for unreliable sexual signals in *Drosophila simulans*. J Evol Biol. 26:311–324.

Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol Rev Camb Philos Soc. 72:283–327.

Lande R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. J Evol Biol. 22:1435–1446.

Lewis SM, Tigreros N, Fedina T, Ming QL. 2012. Genetic and nutritional effects on male traits and reproductive performance in *Tribolium* flour beetles. J Evol Biol. 25:438–451.

Matsubayashi KW, Ohshima I, Nosil P. 2010. Ecological speciation in phytophagous insects. Entomol Exp Appl. 134:1–27.

Messina FJ, Fry JD. 2003. Environment-dependent reversal of a life history trade-off in the seed beetle *Callosobruchus maculatus*. J Evol Biol. 16:501–509.

Messina FJ, Mendenhall M, Jones JC. 2009. An experimentally induced host shift in a seed beetle. Entomol Exp Appl. 132:39–49.

NIST AMDIS, 2005. Automated mass spectral deconvolution and identification system AMDIS 2.64. Released December 2005.Gaithersburg (MD): National Institute of Standards and Technology.

Nosil P. 2012. Ecological speciation. Oxford (UK): Oxford University Press.

Nosil P, Crespi BJ, Gries R, Gries G. 2007. Natural selection and divergence in mate preference during speciation. Genetica. 129:309–327.

Nowicki S, Searcy WA, Peters S. 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 188:1003–1014.

Oyama S. 2000. The ontogeny of information: developmental systems and evolution. Durham (NC): Duke University Press.

Pawlowsky-Glahn V, Buccianti A. 2011. Compositional data analysis: theory and applications. Hoboken (NJ): John Wiley & Sons.

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol. 25:459–467.
Pigliucci M. 2001. Phenotypic plasticity: beyond nature and nurture. Baltimore (MD): The Johns Hopkins University Press.
Qvarnström A, Pärt T, Sheldon BC. 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. Nature. 405:344–347.
Rice WR. 1989. Analyzing tables of statistical tests. Evolution. 431:223–225.
Rodríguez RL, Sullivan LM, Snyder RL, Cocroft RB. 2008. Host shifts and the beginning of signal divergence. Evolution. 62:12–20.
Rolán-Alvarez E, Caballero A. 2000. Estimating sexual selection and sexual isolation effects from mating frequencies. Evolution. 54:30–36.
Ronald KL, Fernández-Juricic E, Lucas JR. 2012. Taking the sensory approach: how individual differences in sensory perception can influence mate choice. Anim Behav. 84:1283–1294.
SAS Institute, Inc. 2010. The SAS System for Windows. Release 9.3. Cary (NC): SAS Institute.
Savković U, Vučković I, Stojković B. 2012. The growth on different stored legume species affects the profiles of cuticular hydrocarbon (CHC) in *Acanthoscelides obtectus* (Say). J Stored Prod Res. 50:66–72.
Schlichting CD, Pigliucci M. 1998. Phenotypic evolution: a reaction norm perspective. Sunderland (MA): Sinauer Associates.
Smadja C, Butlin RK. 2009. On the scent of speciation: the chemosensory system and its role in premating isolation. Heredity. 102:77–97.
Smith G, Fang Y, Liu X, Kenny J, Cossins AR, de Oliveira CC, Etges WJ, Ritchie MG. 2013. Transcriptome-wide expression variation associated with environmental plasticity and mating success in cactophilic *Drosophila mojavensis*. Evolution. 67:1950–1963.
Sokal RR, Rohlf FJ. 1995. Biometry. New York (NY): WH Freeman.
Sokolovska N, Rowe L, Johansson F. 2000. Fitness and body size in mature odonates. Ecol Entomol. 25:239–248.
Stojković B, Šešlija-Jovanović D, Perovanović J. Tucić N. 2011. Sexual activity and reproductive isolation between age-specific selected populations of seed beetle. Ethology. 117:812–821.
Stojković B, Šešlija-Jovanović D, Tucić B, Tucić N. 2010. Homosexual behaviour and its longevity cost in females and males of the seed beetle *Acanthoscelides obtectus*. Physiol Entomol. 35:308–316.
Stojković B, Šešlija-Jovanović D, Tucić N. 2012. Transgenerational effects on overall fitness: influence of larval feeding experience on the oviposition behaviour of seed beetle *Acanthoscelides obtectus* (Say). Pol J Ecol. 60:387–393.
Sullivan MS. 1994. Mate choice as an information gathering process under time constraint: implications for behaviour and signal design. Anim Behav. 47:141–151.
Suzuki Y, Nijhout HF. 2006. Evolution of a polyphenism by genetic accommodation. Science. 311:650–652.
Tregenza T, Buckley SH, Pritchard VL, Butlin RK. 2000. Inter- and intrapopulation effects of sex and age on epicuticular composition of meadow grasshopper, *Chorthippus parallelus*. J Chem Ecol. 26:257–278.
Vanbergen AJ, Raymond B, Pearce IS, Watt AD, Hails RS, Hartley SE. 2003. Host shifting by *Operophtera brumata* into novel environments leads to population differentiation in life-history traits. Ecol Entomol. 28:604–612.
Waddington CH. 1953. Genetic assimilation of an acquired character. Evolution. 7:118–126.
West-Eberhard MJ. 2003. Developmental plasticity and evolution. Oxford (UK): Oxford University Press.

Downloaded from https://academic.oup.com/beheco/article-abstract/25/3/553/511561
by Belgrade University user
on 31 May 2018