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THE MAINTENANCE OF GENETIC VARIATION FOR OVIPOSITION RATE IN TWO-SPOTTED SPIDER MITES: INFERENCES FROM ARTIFICIAL SELECTION

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Despite the directional selection acting on life-history traits, substantial amounts of standing variation for these traits have frequently been found. This variation may result from balancing selection (e.g., through genetic trade-offs) or from mutation-selection balance. These mechanisms affect allele frequencies in different ways: Under balancing selection alleles are maintained at intermediate frequencies, whereas under mutation-selection balance variation is generated by deleterious mutations and removed by directional selection, which leads to asymmetry in the distribution of allele frequencies. To investigate the importance of these two mechanisms in maintaining heritable variation in oviposition rate of the two-spotted spider mite, we analyzed the response to artificial selection. In three replicate experiments, we selected for higher and lower oviposition rate, compared to control lines. A response to selection only occurred in the downward direction. Selection for lower oviposition rate did not lead to an increase in any other component of fitness, but led to a decline in female juvenile survival. The results suggest standing variation for oviposition rate in this population consists largely of deleterious alleles, as in a mutation-selection balance. Consequently, the standing variation for this trait does not appear to be indicative of its adaptive potential.

KEY WORDS: Antagonistic pleiotropy, balancing selection, genetic trade-off, life-history trait, mutation-selection balance, standing variation, *Tetranychus urticae*, *Wolbachia*.

Understanding how additive genetic variation in a population (standing variation) is maintained, is a central issue in evolutionary genetics (Barton and Keightley 2002; Mitchell-Olds et al. 2007; Mackay et al. 2009). For life-history traits, which are directly linked to fitness, one would expect that strong directional selection greatly reduces genetic variability (Charlesworth and Hughes 1999). However, high standing variation is ubiquitously observed in life-history traits in many populations (e.g., Mousseau and Roff 1987; Kruuk et al. 2000; Merilä and Sheldon 2000; Stirling et al. 2002; McCleery et al. 2004). Here, we ask which factors maintain this variation.

Genetic variation is continuously generated by mutation (Lynch and Walsh 1998; Haag-Liautard et al. 2007), but most mutations are deleterious (e.g., Fay et al. 2001; Sawyer et al. 2007) and partially recessive, causing fitness to decrease by 1 or 2% in the heterozygous state (Simmons and Crow 1977; Houle et al. 1994). Deleterious mutations are eventually eliminated by purifying selection, but persist on average for tens of generations

(Lynch and Walsh 1998, Ch. 12, p. 351–352). An equilibrium between the influx through mutation and removal by selection is called a mutation-selection balance and leads to the presence of genetic variation in the population; however, shortly individual alleles may persist. Standing variation can also arise if selection actively maintains polymorphisms within a population, i.e., balancing selection. This may occur via direct selection, for example, if the relationship of a trait with fitness is frequency-dependent or fluctuates temporally or spatially (Barton and Keightley 2002). Since life-history traits are directly related to fitness, the most likely scenario for balancing selection working on a life-history trait involves indirect selection, where the trait has a negative genetic relationship with other fitness-determining traits.

The forces that maintain additive variation in a trait also shape its genetic architecture (Barton and Keightley 2002; Mitchell-Olds et al. 2007). Standing variation caused by balancing selection is intrinsically different from variation originating from a mutation-selection balance. Alleles maintained by balancing selection exist at intermediate frequencies, whereas in a mutation-selection balance variation arises from rare alleles (Turelli and Barton 2004; Johnson and Barton 2005). In the latter case, most genetic variation will be for trait values lower than the mean, while under balancing selection variation will be distributed more symmetrically around the mean. We propose to use this difference in the allele frequency distribution to investigate the relative importance of the two mechanisms, via artificial selection in both directions. The level of asymmetry in selection response depends on the divergence of the initial gene frequencies away from the midpoint between extreme phenotypic values (Falconer and Mackay 1996, Ch. 12, p. 212–213). If additive variation is maintained purely by a mutation-selection balance, selection for higher phenotypic values should trigger only a weak response, since selectable variation exists mainly for lower phenotypic values. If balancing selection is an important factor, then a response to artificial selection should readily occur in both directions.

Research on the relative importance of balancing selection and mutation-selection balance in explaining the standing variation is scarce and has focused on *Drosophila melanogaster* (Houle et al. 1994; Fry et al. 1998; Charlesworth and Hughes 1999; Rodriguez-Ramilo et al. 2004; Charlesworth et al. 2007; Long et al. 2009) and to a lesser extent on the plant *Mimulus guttatus* (Kelly and Willis 2001; Kelly 2003). We worked with the two-spotted spider mite, *Tetranychus urticae* Koch, a polyphagous herbivore with a haplodiploid genetic system, a high intrinsic growth rate, and a remarkable investment in dense webs of silken threads that serve as a defense against predators (Helle and Sabelis 1985). In a natural population of *T. urticae,* the early-life oviposition rate was found to exhibit relatively high narrow-sense heritability ($h^2 = 0.75$) and additive genetic variation ($CV_A = 28$), when compared with other life-history traits within the population and with similar traits in other species (Tien et al. 2009). This triggers the question why there is so much standing variation for this trait. Here, we performed artificial selection on the oviposition rate of *T. urticae* in order to explore this question. We used a long established laboratory population in order to rule out laboratory adaptation during the experiment. The response to upward and downward selection was examined in three replications, each with control lines. Correlated responses in seven fitness traits were examined. If genetic trade-offs play a role, then opposing effects may be found in other fitness traits in the subsequent selection lines (although these effects need not necessarily emerge; Pease and Bull 1988; Charlesworth 1990). We also determined the narrow-sense heritability of oviposition rate in this population through a mother–daughter regression analysis.

To eliminate another potential cause for inherited variation, we checked for the presence of maternally inherited cytoplasmic bacteria. *Tetranychus urticae* is a known host for *Wolbachia* (Breeuwer and Jacobs 1996) and *Cardinium* (Gotoh et al. 2007), which are transmitted from mother to offspring via the cytoplasm and can induce reproductive alterations in their host, such as a change in oviposition rate (Vala et al. 2000). Thus, an inherited maternal effect of the infection on oviposition rate may exist. Also, infection is often not fixed in the population (i.e., not all individuals are infected) (Hoffman et al. 1990). If oviposition rate varies with infection status and the infection is not fixed, then variation in oviposition rate may not be related to the genetics of the spider mite, yet nevertheless maternally inherited. In order to distinguish between additive genetic and maternally inherited bacterial variation, we determined the patterns in infection status with respect to *Wolbachia* and *Cardinium* and estimated the correlation with oviposition rate.

Materials and Methods

The laboratory population of *T. urticae* was collected in 1994 in a commercial greenhouse in Pijnacker, The Netherlands. The mites were reared on cucumber (*Cucumis sativus*, variety Ventura) (>10,000 individuals) and before the start of the experiment at least 10 generations (with more than 500 individuals at any time) on common bean (*Phaseolus vulgaris*), which was also the host plant in our experiment. Bean is a benign host plant, on which the oviposition rate of *T. urticae* is at least as high as on cucumber (M. Egas, unpubl. data). Rearing and experiments took place in a climate-controlled room at 23°C, 60% humidity, and a 16L:8D photoperiod. All statistical analyses were performed with the software "R" (R Development Core Team 2009).

HERITABILITY: MOTHER–DAUGHTER REGRESSION

A cohort of offspring of one female was reared on individual leaf discs (diameter = 1.5 cm). From this cohort, a female in the last

pupal phase was separated and mated with an unrelated male. At age 15 (days since egg was laid) the female (the "mother") was placed on a fresh leaf disc. After 24 hours, the female was removed and her eggs were counted. The procedure of mating and oviposition was repeated with one of her female offspring (the "daughter"). Linear regression was performed of the oviposition rate of the daughters on that of the mothers ($n = 95$). Narrow-sense heritability is estimated as twice the slope of the regression line (see Falconer and Mackay (1996, Ch. 10, p. 163) for general theory and Liu and Smith (2000) for theory specifically for haplodiploid species), with a standard error equal to twice the standard error of the slope (Roff 1997, Ch. 2, p. 46). To determine whether a nonlinear relationship was more appropriate, the degree of fit (r^2) of the linear regression model was compared to that of polynomial regression models, with a quadratic function and with an exponential function.

SELECTION

Selection lines were set up in three replicates, which all consisted of three treatments; a line selected for higher oviposition rate ("high line"), a line selected for lower oviposition rate ("low line") and an unselected line ("control line"). The three lines within a replicate were treated similarly, except for the selection regime. The main differences between the replicates were the dates of the experimental steps (and thus the batch of plants they were offered as food), and the age of the females at which the oviposition rate was measured. The age at oviposition assessment varied randomly between 14 and 18 days in every selection round for every replicate. This was done to avoid selection on oviposition rate at a specific age and to maintain selection on early-life oviposition rate. Six selection rounds were realized for all replicates, except for the low line and the high line of replicate 2, which had five and four selection rounds, respectively (due to unknown methodological problems in round 4 for replicate 2 and start-up problems for high line 2 [see below]). For the selection lines, selection by truncation took place at the 33% level. The selected population consisted of c. 50 out of 150 females. To select individuals, 150 (mated) females were kept on individual leaf discs for 48 hours after which the eggs were counted and the 50 females with the most (for the high lines) or the least (for the low lines) eggs were selected. If a female was selected, 12–15 of her eggs were transferred to a bean leaf, which contained the eggs of all selected females of that line. After reaching adulthood (and having mated), 150 randomly picked females were tested again. In the control line, the same protocol was used except that 50 females were picked at random in every round. After the oviposition rate of the females in the last selection round was determined, populations were set up for each line and these were provided with ample food and kept at a population size of at least 200 adult females. The populations were maintained in this manner until all consecutive work was completed.

Because in the first selection round, less than 12 eggs were collected, in three lines (low line 1 and 2, high line 2) not enough adult females were available after the first selection round. Since only one round of selection had occurred and phenotypic differences between lines were still small, the females were put together with females from another low line (in the case of low line 1) or from the control line (in the case of low line 2). The selection differential and selection response for that round were corrected accordingly. For high line 2 no selection took place in the second round. The number of selected females in these cases was at least 50.

The selection differential is the difference in trait value between the whole population (the 150 females) and the selected part of the population (the 50 selected females). The selection response is the average trait value of the 150 females in the next generation of the selection line minus the average trait value of the next generation of the control line. By taking values relative to those of the control lines, variation due to environmental fluctuations is reduced. Linear regression with a normal error distribution was performed for the selection response on the selection differential. The realized heritability (h_r^2) is then twice the slope of the regression line (b), with $SE(h_r^2) = 2xS.E(b)$ (see Falconer and Mackay (1996, Ch. 11, p. 191) for general theory and see Margolies and Cox (1993) and Orzack and Gladstone (1994) for application to haplodiploids. Note that—to the best of our knowledge—formal theory for haplodiploids is still lacking).

The effect of the selection regime on the amount of phenotypic variation in oviposition rate was examined using a linear mixed effect model (Crawley 2007, Ch. 19, p. 627–660). The standard deviation within a line for each generation was taken as a measure for the amount of variation. Data from the three replicates were grouped to obtain a dataset large enough for appropriate modeling. Selection regime (high/low/control), selection round (as continuous variable, with values from 0 to 6), and their interaction were incorporated as fixed factors and the selection round within each line was incorporated as random factor to avoid pseudoreplication. Model simplification was performed in order to reach the minimal adequate model, in which all factors and factor levels are significant (Crawley 2007, Ch. 13, p. 325–329). Nonsignificant fixed factors were removed from the model (i.e., if their removal from the model did not cause a significant increase in deviance) and factor levels that did not differ from each other were merged into one level.

CORRELATED RESPONSES

The fitness traits that were measured in the nine lines were (1) juvenile development rate, (2) juvenile survival, (3) number of adult sons, (4) number of adult daughters, (5) fecundity, (6) longevity, and (7) web production. The eggs counted after the last selection round were used to measure the life-history traits. On the 13th day

the development rate, juvenile survival and number of sons and daughters were scored. To quantify development rate, an index was created of the average stage of offspring, where all individuals were scored with regard to their stage, as in Tien et al. (2009): adult = 7, teleiochrysalis = 6, deutonymph = 5, deutochrysalis = 4, protonymph = 3, protochrysalis = 2, larva = 1. Juvenile survival was calculated as the fraction of offspring still alive on the 13th day. Sons and daughters were counted in the offspring that had reached the teleiochrysalis or adult stage. Although not all offspring had reached these stages yet at that age, the sex ratio was similar to the final sex ratio in another experiment with these populations, where all individuals were allowed to mature (in the experiment on bacterial effects, data not shown). The same argument holds for juvenile survival: out of the offspring that reached this age, 97% eventually reached adulthood, implying survival to this age is a reliable estimate for total juvenile survival (data not shown). One newly emerged virgin adult daughter was placed on a fresh leaf disc to measure longevity and fecundity. Every couple of days, the eggs were counted. The leaf disc was refreshed c. once per week, until the female died. Longevity was measured as the number of days of adult life and fecundity as the lifetime number of eggs. For web production, the protocol of Tien et al. (2009) was used: Females (18 days old) were placed on a leaf disc (with a 1.5 cm diameter) put upside-down on wet cotton wool and cut from first leaves of two-week-old bean plants with the main vein of the leaf running down the center of the disc. Only two discs per leaf were cut, nearest to the basis of the vein, to ensure that leaf discs exhibited veins of similar size and structure. After 24 hours c. 500 sand particles (with equal shape and size [63–90 μm]) were sprinkled over the disc with a fine brush, covering the surface homogeneously. The 3D structure of the web ensures that some particles land on the silk and others on the leaf surface. The numbers of particles on the leaf surface and in the silken threads of the web were counted. Web production was defined as the ratio of the number of particles in the web over the total number.

Since no response to selection for higher oviposition rate was found (see Results section), the correlated responses were only analyzed for the lines selected for lower oviposition rate. The three replicates were taken as independent experiments and analyzed separately, with treatment as a fixed effect. GLMs with appropriate error distributions were performed per replicate, with selection regime as the explanatory variable. An appropriate test (F or Chi-square) was subsequently performed to compare this model to the null model (Crawley 2007, Ch. 9). Development rate was transformed into log(7-X) and analyzed assuming a normal error distribution. The fecundity data of replicate 1 and 2 were analyzed assuming a normal error distribution, but the data of replicate 3 did not comply with a normal error distribution. Therefore, these data were analyzed with a nonparametric Kruskal-Wallis test. As a control, the fecundity data of replicate 1 and 2 were also analyzed with a Kruskal-Wallis test, which yielded similar *P*-values as in the GLM. The number of sons and daughters were analyzed assuming a Poisson error distribution. The juvenile survival data were analyzed assuming a binomial error distribution, except if there was overdispersion (i.e., if the residual scaled variance was more than three times larger than the residual degrees of freedom), in which case a quasibinomial error distribution was assumed in order to scale the variance appropriately (Crawley 2007, Ch. 16, p. 327–329). The longevity data were analyzed with the Kruskal-Wallis test.

CROSSES

Matings were set up for every replicate between individuals from the high line ("high cross"), from the low line ("low cross"), and in a combination of the two lines; between a female from the high line with a male from the low line ("reciprocal cross 1") and between a female from the low line with a male from the high line ("reciprocal cross 2"). Teleiochrysalis females were placed on a leaf with newly emerged males. Once mature, the females were placed on individual leaf discs for a day to oviposit. When this offspring reached the teleiochrysalis stage, the males were changed between leaf discs within a treatment to exclude potential inbreeding effects by brother–sister mating. When 19 days old, the females were placed on new leaf discs and their oviposition rate was measured over 48 hours. The sample sizes were: 25, 31, and 30 for the high cross; 28, 29, and 31 for the reciprocal cross 1; 24, 30, and 30 for the reciprocal cross 2; and 26, 30, and 29 for the low cross. The experiment was set up circa 13 generations after ending the selection regime. An analysis of variance (ANOVA) model was used with the crossing treatment and the replicate as independent variables and oviposition rate as dependent variable.

BACTERIAL EFFECTS ON OVIPOSITION RATE

Egg cohorts were produced for all lines, c. 12 generations after ending the selection regime. Individual adult females were placed on leaf discs and two days later the number of eggs was counted and the females were frozen at −80°C until used in the PCR procedure. After 13 days and every day thereafter, the emerged adult males and females were counted, until all offspring had reached adulthood or had died. DNA was extracted from single mites using a Chelex extraction procedure (Groot and Breeuwer 2006). Supernatant (2 μl) was used as template per 10 μl reaction. The presence of DNA of *Wolbachia* and *Cardinium* was determined using PCR amplification of the genes *wsp* and *gltA* (*Wolbachia*) and 16S rDNA and *gyrB* (*Cardinium*) (Ros and Breeuwer 2009). As a positive control for DNA extraction, part of the mitochondrial COI gene of the spider mite was amplified (Ros and Breeuwer 2009). *Cardinium*-infection was not found in the first 48 females tested (in replicate 2 and 3) and no more individuals were screened

for *Cardinium*. For *Wolbachia*-infection, 36 females per replicate per control line were screened (totaling 108 females), and 20 per selection line (totaling 120 females). G-tests were used to determine the difference in *Wolbachia* occurrence between lines. The difference in average trait value between *Wolbachia*-infected and uninfected females was determined in the three control lines, for oviposition rate (number of eggs per 48 hours), juvenile survival (fraction of offspring that survived from egg to adult), maturity rate (fraction of adult offspring that had reached adulthood after 13 days), and total number of adult males and females. GLMs with appropriate error distributions were performed per trait, with *Wolbachia*-infection status (present/absent), replicate, and the interaction of infection status and replicate as explanatory variables.

Results

NARROW-SENSE HERITABILITY

The mother–daughter linear regression revealed significant narrow-sense heritability of $h^2 = 0.47$ (SE(h^2) = 0.19, $F_{1,93} = 5.82$, $P = 0.018$). The coefficients of variation amounted to $CV_A = 15.01$ and $CV_R = 15.94$. The phenotypic frequency distributions per generation and the residuals of the regression model indicated a normal error distribution and homogeneity of variance (see Appendix S1). Also, using nonlinear models did not improve the fit of the model; the degree of fit (r^2) did not increase in a polynomial regression model with a quadratic function or an exponential function.

SELECTION

In all three replicates, selection for higher oviposition rate did not result in a response; the oviposition rate did not increase compared to the control lines (Fig. 1, Table 1, Appendix S2). A response to selection for lower oviposition rate occurred in all three replicates. The selection differential significantly influenced the selection response in the low lines 1 and 2 (Fig. 1, Table 1, Appendix S2). In low line 3, the response was not significant ($P = 0.10$), but the final oviposition rate of the low line was lower than that of the control line (difference = −3.15 eggs per 48 hours, ANOVA; $F_{1,92} = 34.2$, $P < 0.001$) and remained so

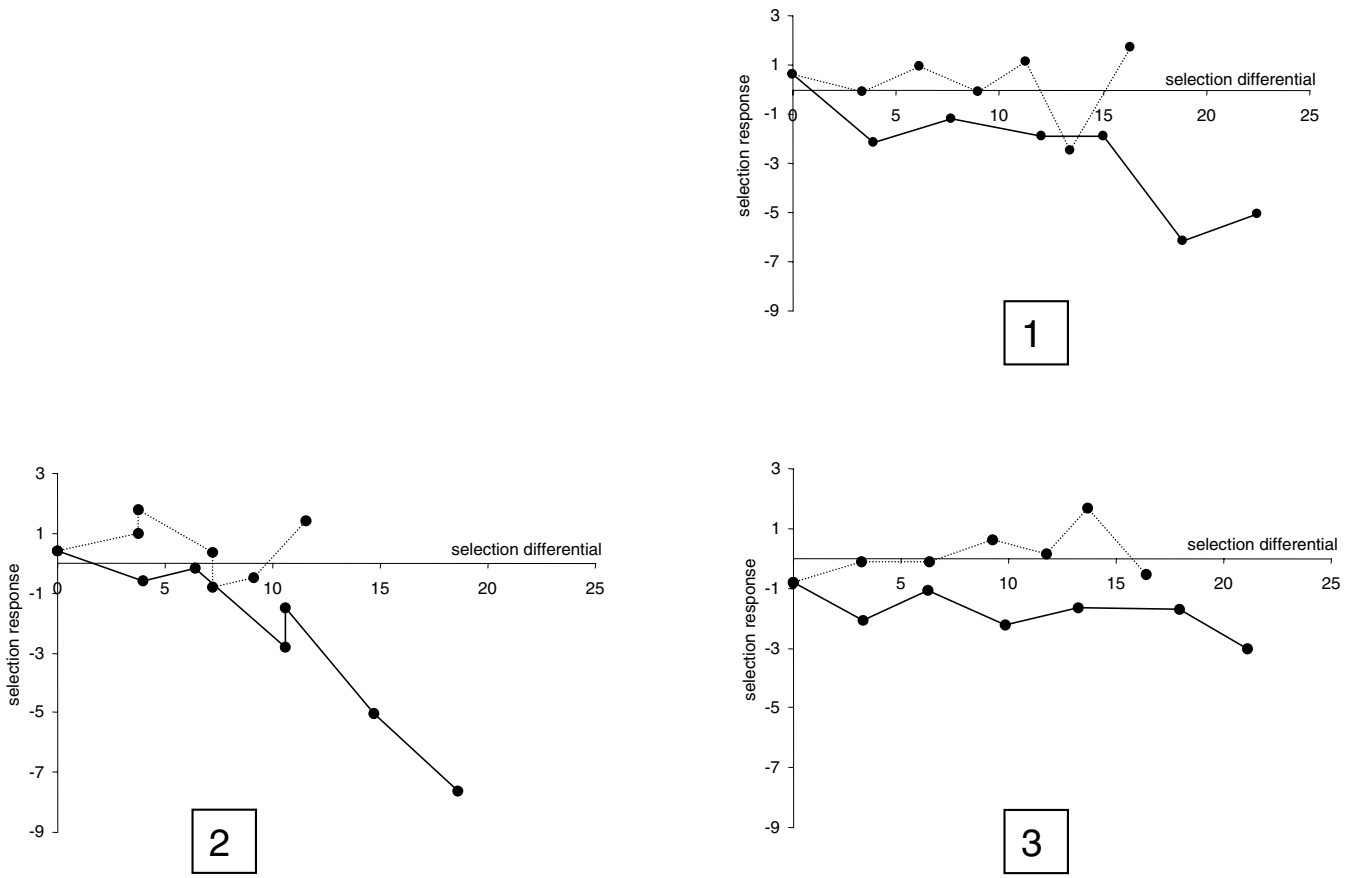

Figure 1. Selection response of oviposition rate (no. of eggs in 48 hours) to upward and downward selection plotted against the cumulative selection differential for that selection round, for the three replicates (1, 2, and 3). The selection response is relative to the oviposition rate of the control line. The response to upward selection is shown with a dashed line, the response to downward selection with a solid line.

Table 1. Realized heritabilities (h_r^2) for the six selection lines. "*P*" is the *P*-value of the linear regression model, "SE (h_r^2)" is the standard error of the realized heritability. "Mean h_r^2" is the average of the realized heritabilities of the three low lines, with its standard error="SE (mean h_r^2)".

Replicate	Increased oviposition rate (high line)			Decreased oviposition rate (low line)				
	P	h_r^2	SE (h_r^2)	*P*	h_r^2	SE (h_r^2)	Mean h_r^2	SE (mean h_r^2)
1	0.89	−0.02	0.11	0.01	0.48	0.14		
2	0.77	−0.04	0.11	0.002	0.86	0.14	0.49	0.21
3	0.31	0.07	0.06	0.10	0.14	0.06		

up to 13 generations after selection ended (data from the crossing experiments and the experiment on bacterial effects; results not shown). The mean realized heritability for the three low lines was 0.49 (Table 1).

The phenotypic frequency distributions remained normal throughout the selection procedure (Appendix S3), but the amount of phenotypic variance did change: The effect of selection regime, selection round, and their interaction on the amount of variance were analyzed and this led to the following results. Selection regime by itself had no effect (log-likelihood ratio = 2.4, $P = 0.30$) and was removed from the model, but selection round and the interaction between selection regime and selection round were significant factors. The slope of the regression line through the selection rounds of the high and control treatments were not significantly different from each other ($t = -1.35$, $P = 0.13$) and the two factor levels of the interaction were merged into one ("control-high"). In the subsequent minimal adequate model, selection round ($t = -5.5$, $P < 0.01$) and the interaction of selection round with selection regime (low vs. control-high) ($t = 5.0$, $P < 0.001$) were significant factors. With regards to the interaction, the slope of the regression line for the low treatment over the selection rounds was larger than that of the control-high treatment, with a relative slope of $b = 0.17$ ($\pm$SE $= 0.03$). To determine the relative slope over the (more meaningful) selection differential, a similar mixed effect model was analyzed with selection differential (instead of selection round), selection regime (high vs. low), and their interaction as explanatory variables. This yielded similar results and a positive slope of the low treatment (relative to the high treatment) over the selection differential, equal to $b = 0.08$ ($\pm$SE $= 0.01$).

CORRELATED RESPONSES

Selection for lower oviposition rate did not lead to an (significant or trend-wise) improvement in any of the examined other life-history traits or web production (Table 2). The only consistent correlated responses were toward lower trait values: The juvenile survival of the low lines was lower than that of their control lines, on average 19% lower (Table 2). Juvenile death took place for 73% in the egg stage (results not shown). Also, the number of daughters in the pre-adult (pupal) and adult stage was consistently and significantly lower in all low lines with an average reduction of 71%. The number of sons, longevity, and development rate were not affected. For fecundity and web production, differences between the control and low lines were not consistently significant across the three replicates ($P < 0.05$ in two out of three replicates in both cases), but the low lines had lower trait means in all replicates. Since selection for increased oviposition rate yielded no response, no correlated responses in other fitness traits were expected or found; no consistent change (compared to the control line) was present in any trait across the three replicates of the high lines (results not shown).

CROSSES

Crosses between the high and low line of each replicate were set up to confirm the genetic basis of the differences between the lines and to evaluate the potential presence of maternal and/or paternal effects. In order to exclude maternal or paternal effects, the oviposition rates of the reciprocal crosses should be equal to each other and equal to the average of those of the high and the low cross. There was no difference between replicates ($F_{2,179} = 1.46$, $P = 0.23$), but the crossing treatment led to significant differences in oviposition rates ($F_{3,179} = 24.72$, $P < 0.001$, Fig. 2). When reanalyzing the data with only the crossing treatment as explanatory variable, the oviposition rate of the two reciprocal crosses did not differ from each other (Tukey's HSD post hoc test, $P = 0.9995$), but all other combinations were significantly different (all $P < 0.05$). Also, the oviposition rates of the two reciprocal crosses were both intermediate to the oviposition rates of the two parental crosses (i.e., the dashed line in Fig. 2); in one-sample *t*-tests, the oviposition rates of the reciprocal crosses did not differ from being intermediate to the two parental crosses ($t_{44} = -0.61$, $P = 0.5$ and $t_{44} = -0.49$, $P = 0.6$ for reciprocal cross 1 and 2, respectively. The data for the two reciprocal crosses were first normalized by subtracting the mean of the high and low crosses [i.e., the dashed line in Fig. 2] from each sample).

Table 2. Descriptive statistics of the correlated responses to artificial selection. The mean value (except for longevity; median value), its standard error (SE), the sample size (*N*), and the *P*-value (*P*-values shown in bold if <0.05) are shown, for the traits juvenile survival (survival from egg to adult), juvenile development rate (average development stage of all offspring at a specific age), number of adult sons, number of adult daughters, web production, fecundity (number of eggs laid over a lifetime), and longevity (days lived as an adult).

	Replicate 1		Replicate 2		Replicate 3	
	Low	Control	Low	Control	Low	Control
Juvenile survival	0.63	0.86	0.66	0.80	0.79	0.90
SE	0.04	0.02	0.03	0.02	0.03	0.02
N	63	40	47	45	47	47
P	**<0.001**		**<0.001**		**0.007**	
Develop. rate	5.36	5.60	5.83	5.85	4.88	5.78
SE	0.08	0.05	0.08	0.04	0.07	0.03
N	61	41	48	45	45	47
P	0.12		0.6		**<0.001**	
No. sons	3.56	3.37	3.65	4.07	2.17	2.60
SE	0.40	0.41	0.37	0.45	0.25	0.28
N	52	41	46	44	30	47
P	0.6		0.3		0.2	
No. daughters	1.98	6.44	3.24	8.93	1.73	8.64
SE	0.26	0.58	0.40	0.52	0.37	0.51
N	52	41	46	44	30	47
P	**<0.001**		**<0.001**		**<0.001**	
Web production	20.89	23.83	13.69	21.47	30.72	36.66
SE	2.15	1.57	1.06	2.61	1.83	1.41
N	12	10	10	10	10	10
P	0.14		**0.01**		**0.008**	
Fecundity	83.20	119.92	61.17	157.87	137.00	163.09
SE	13.46	6.49	10.74	13.32	12.09	12.73
N	15	25	23	23	21	23
P	**0.009**		**<0.001**		0.09	
Longevity	23	19	33	30	19	20
N	15	25	23	23	21	22
P	0.3		0.3		0.8	

CYTOPLASMIC BACTERIA

No *Cardinium* infection was found in the first 48 females tested, which led us to conclude that *Cardinium* infection was too rare to have had a relevant effect. *Wolbachia* infection was present at intermediate levels in all three control populations (Table 3). On average, 56% of the females in the control lines were infected with *Wolbachia.* However, oviposition rate did not differ with infection status ($P = 0.35$). It also did not differ with the interaction between infection status and replicate line ($P = 0.59$), but only between replicate lines ($P < 0.001$). Infection with *Wolbachia* also did not affect any other life-history trait (Table 3). In addition, no consistent difference in infection frequencies was found between the low lines and their control lines (infection in the low lines: 15%, 0%, and 100% for replicates 1, 2, and 3, respectively, with $N = 20$. See Table 3 for data on the control lines.). If *Wolbachia*-infection had affected the oviposition rate, a consistent difference in infection frequency is expected after artificial selection on oviposition rate, since infection would indirectly be under selection. The absence of such consistent differences complies with the finding that the *Wolbachia*-infection does not appear to influence the oviposition rate of *T. urticae* in this population.

Discussion

In two populations of *T. urticae*, we found high narrow-sense heritability and CV_A for oviposition rate using a mother–daughter breeding design (this article and Tien et al. 2009). This result prompts the following questions: Why does this variation for oviposition rate, being a fitness determinant, exist in the face of directional selection? Are endosymbiotic bacteria responsible for maternally transmitted inherited variation? Does balancing selection preserve genetic variation? And/or does mutation cause a large pool of transient deleterious alleles?

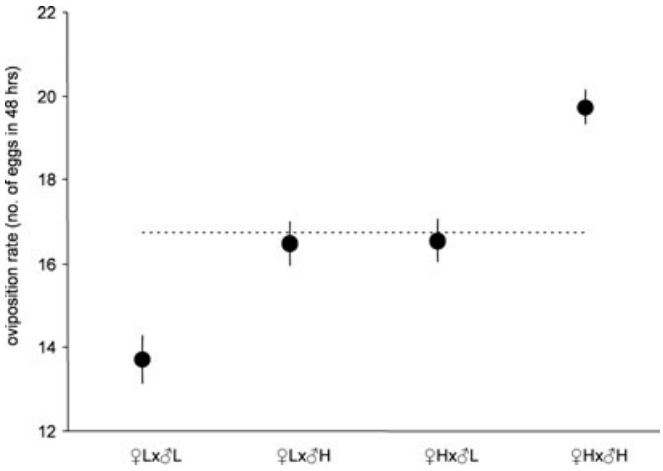

Figure 2. **Mean oviposition rate (and standard error) of crosses, averaged over all replicates. H, high line; L, low line. The two reciprocal crosses between the high line and the low line are not significantly different ("ns") from each other; all other combinations are significantly different. The dotted line is the average of the oviposition rates of the high cross and the low cross.**

NO HERITABLE EFFECTS VIA CYTOPLASMIC BACTERIA

Cardinium was found to be absent, but *Wolbachia* occurred in the three control lines at intermediate infection frequencies (on average 56%). This satisfies the first condition for *Wolbachia*-related inherited variation, namely a population with a heterogeneous infection status. However, the oviposition rate of *Wolbachia*-infected mites was equal to that of uninfected mites and thus the infection status cannot have caused the observed similarity in oviposition rate between mothers and daughters nor the subsequent heritability estimates. Also, no evidence for indirect selection on *Wolbachia* was found, since there were no consistent differences in infection frequency of the low lines compared to their control lines. Another indication for the absence of overall maternal effects comes from the crossing results: When crossing the high and low lines, the offspring of a female of the high line (reciprocal cross 1) had a similar oviposition rate to that of a female of the low line (reciprocal cross 2).

Although cytoplasmic bacteria were present in the population under study, they did not influence the oviposition rate. It should, however, be noted that *Wolbachia* and *Cardinium* influence reproductive traits in many arthropod and nematode species (Stouthamer et al. 1999). Potential maternal inheritance via these bacteria should be taken into account when extracting heritability estimates by looking at similarities between relatives. When using a mother–daughter breeding design, the effect could be misinterpreted as an additive genetic effect, but when using a sib/half-sib breeding design, the heritable nature of the maternal effect might be overlooked.

ASYMMETRIC RESPONSE TO SELECTION

Selection for decreased oviposition rate proceeded rapidly and after six selection rounds the oviposition rate of the low lines was on average 71% of that of the control lines. However, upward selection did not lead to increased oviposition rate in any of the lines. This difference in oviposition rate between the lines was genetically determined, as confirmed in the crossing results. How can the asymmetry in response be explained? Falconer and Mackay (1996, Ch. 12, p. 211–214) list eight potential causes for an asymmetric response to selection: Random drift, inbreeding depression, maternal effects, genes of large effect, scalar asymmetry, indirect selection, asymmetric effective selection differentials,

Table 3. **Descriptive statistics of life-history traits of *Wolbachia* infected ("Wolb") and uninfected ("uninf") females of the control lines. The sample size (*N*) and the means with their standard error are shown, for oviposition rate (no. of eggs per 48 hours), juvenile survival (survival from egg to adult), maturity rate (fraction surviving offspring that has reached adulthood at a specific age), number of adult sons and number of adult daughters. No significant differences were found between uninfected and infected females in any trait.**

	Replicate 1		Replicate 2		Replicate 3	
	Uninf	Wolb	Uninf	Wolb	Uninf	Wolb
N	10	26	18	18	19	17
Oviposition rate	19.80	20.50	17.17	16.94	19.84	21.00
SE	0.47	0.52	0.81	0.85	0.61	0.63
Juvenile survival	0.93	0.86	0.74	0.81	0.87	0.90
SE	0.02	0.03	0.06	0.06	0.03	0.04
Maturity rate	0.96	0.88	0.40	0.44	0.55	0.49
SE	0.02	0.03	0.04	0.06	0.04	0.04
No. sons	4.90	4.65	7.44	6.78	5.57	5.53
SE	0.75	0.59	1.22	0.92	0.88	1.02
No. daughters	13.40	13.00	6.11	6.94	11.84	13.53
SE	1.01	1.05	1.25	0.94	1.26	1.19

and genetic asymmetry. Below we explain point by point why six of these mechanisms are not likely as explanations for our data.

First, the use of three replicates and the similar patterns found among them make random drift an unlikely explanation. Second, we corrected for an effect of inbreeding depression (and environmental trends) by using control lines as reference points for the selection lines. Third, maternal effects can be ruled out based on the crossing results we obtained (see Fig. 2). Also, a maternal effect would have only forced a redefinition of the character under selection and is therefore not of interest here. The fourth and fifth cause are scalar asymmetry and genes of large effect, but these two causes can be ruled out due to the linearity of the mother–daughter regression and the normality of the phenotypic frequency distributions in the original population (see the Results section on narrow-sense heritability and Appendix S1). Also, the phenotypes remained normally distributed around the mean throughout the selection procedure (Appendix S3), which implies scaling effects also did not manifest later on. Sixth, Falconer and Mackay list indirect selection. However, selection was directly on oviposition rate and not on a (potentially inappropriate) proxy.

The remaining two mechanisms may both have played a role in our system. Asymmetry in the effective selection differential is implicated by the finding of asymmetric responses to the first round of selection; in replicate 1 and 3 the response to one round of downward selection was significantly larger than to one round of upward selection (Welch t-tests; $t_{296} = -4.7$ & $P < 0.001$, $t_{33} = -0.01$ & $P = 0.99$, $t_{295} = -5.7$, $P < 0.001$, for replicate 1, 2 and 3, respectively. The data were first normalized to the average of their control line and the low line data (x) were transformed to ($-$x). See also Fig. 1.). This cannot be explained by an effect of a genetic asymmetry, because its effect on the selection response depends on a differentiation in gene frequencies and thus does not appear in the first few generations. Although the calculated selection differentials of the high and low selection treatment were roughly equal in the first round of selection, for various reasons the effective selection differentials may not have been similar to the calculated ones. If the effective selection differential of the downward selection treatment was larger than that of the upward treatment, this can cause asymmetric selection responses. This may have happened, even though the three listed causes for this mechanism (Falconer and Mackay 1996, Ch. 12, p. 212) are not likely in our system: (a) variance in oviposition rate was independent of the mean (as deduced from the parent–offspring regression model), (b) from every selected female an equal number of offspring was taken to form the next generation, which excludes fertility as a possible factor, (c) natural selection is not expected to directly promote downward selection on oviposition rate because this trait has a positive relation to fitness.

If asymmetry in the selection differential played a role, it cannot be the sole mechanism explaining the results, since "asymmetry of realized heritabilities cannot be attributed to any cause operating through the selection differential" (Falconer and Mackay 1996, Ch. 12, p. 212) and the asymmetry in the realized heritabilities was pronounced (see Table 1). Therefore, an additional explanation must be sought. The eighth and last potential cause for asymmetric selection responses is genetic asymmetry. If less genetic variation is available for above-average values than for below-average values, a response to upward selection will be slower than to downward selection (Falconer and Mackay 1996, Ch. 12, p. 212–213). Genetic asymmetry can explain the asymmetric realized heritabilities in our system and thus likely contributed to the asymmetry in selection responses. The consistent extremity of the selection asymmetry here (no response to upward selection within six selection rounds) suggests a strong genetic asymmetry.

BALANCING SELECTION VERSUS MUTATION-SELECTION BALANCE

Our results do not support the scenario where balancing selection is a central mechanism explaining the standing variation in oviposition rate. This is because balancing selection would lead to variants at intermediate frequencies (Turelli and Barton 2004; Johnson and Barton 2005), which should therefore allow a response to selection in both directions. Balancing selection through direct selection on the trait was not assumed to have acted on the oviposition rate, because this trait is supposedly under continuous directional selection. However, our results also do not point toward a leading role of balancing selection involving indirect selection through antagonistic pleiotropy. This is supported by the absence of genetic trade-offs with any of the investigated life-history traits or web production. Although the list of investigated fitness traits is incomplete, it does include many of the most important life-history traits, namely, fecundity, juvenile survival, development rate, longevity, and number of adult offspring. Also, the high investment in defense through the production of silk webbing (Tien et al. 2009) does not seem to trade-off with reproduction.

Given the lack of response to upward selection and the exclusively positive genetic correlations with other life-history traits, the standing variation appears to be caused by genotypes that are in all aspects less fit. Neither balancing selection nor maternally inherited cytoplasmic bacteria can explain these observations. Hence, the question arises whether a mutation-selection balance can account for the data. Three findings are in agreement with this hypothesis. First, the strong response to downward selection and the lack of a significant response to upward selection are consistent with the asymmetric allele frequency distributions expected under mutation-selection balance. Second, the exclusively positive genetic relationships with other life-history traits are in agreement with a scenario where variation is caused by

mutations, since mutations tend to have negative effects on more than one life-history trait (Simmons and Crow 1977; Houle et al. 1994). A positively correlated response to selection was found for juvenile survival and number of daughters. It is likely that this involves one trait, namely female juvenile survival, in particular in the egg stage, since 73% of all juvenile mortality occurred in this stage. Third, the phenotypic variation increased through the selection rounds in the downward selection treatment (relative to the control treatment), while the variation in the upward selection treatment remained equal to that in the control treatment. This process is as expected when variation in a population is caused by deleterious alleles that are initially rare but increase in frequency under downward selection. It should be noted, however, that a unidirectional increase in variation is also one of the possible outcomes if variation is caused by alleles at intermediate frequencies (Kelly 2008) and is as such not discriminating between the scenarios under test in this article.

In conclusion, we have found no evidence for a dominant role of balancing selection as explanation for the high standing variation in oviposition rate. Instead, the data suggest that new mutations contribute substantially to the genetic variation for this fitness trait of *T. urticae*. Whether this holds for other populations remains to be investigated: In order to exclude the interference of laboratory and host adaptation during artificial selection, we used a long established laboratory population of *T. urticae* and a host plant commonly considered to be benign. The lack of response to upward selection confirms the absence of host or laboratory adaptation during the experiment. However, we do not know whether in a population under environmental heterogeneity (instead of the more constant environment of the laboratory), balancing selection via genetic trade-offs does play a role. If a trade-off exists between oviposition rate and another fitness trait and the importance of each trait for fitness differs between environmental conditions, then selection could be balancing in the field. Once in the laboratory, this genetic variation would be lost under the more constant environmental conditions.

Quantitative genetical research on the relative importance of balancing selection and mutation-selection balance for life-history traits has shown that the contribution of either mechanism differs per population and species (Houle et al. 1994; Fry et al. 1998; Lynch et al. 1998; Kelly and Willis 2001; Kelly 2003; Rodriguez-Ramilo et al. 2004; Charlesworth et al. 2007; Long et al. 2009) and between life-history traits within a population (Houle et al. 1994; Fry et al. 1998). If a mutation-selection balance is the main factor determining the standing variation in a population, this poses a problem for the interpretation of parameters of additive genetic variation such as h^2 and CV_A. A significant share of deleterious mutations in the standing variation diminishes the potential of this variation as material for long-term evolution (Mitchell-Olds et al. 2007). Also in our system the presence of additive genetic variation does not appear to have predictive power for the adaptive potential of the trait.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Plots regarding the analysis of the mother–daughter resemblance.

Appendix S2. Oviposition rate (mean and its standard error) over the selection rounds of the three lines per replicate.

Appendix S3. Frequency distribution of the oviposition rate of the two selection lines after the fifth round of selection and of the base line (before selection, round 0).

Supporting Information may be found in the online version of this article.

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