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Adaptation in a spider mite population after long-term evolution on a single host plant

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Abstract

Evolution in a single environment is expected to erode genetic variability, thereby precluding adaptation to novel environments. To test this, a large population of spider mites kept on cucumber for approximately 300 generations was used to establish populations on novel host plants (tomato or pepper), and changes in traits associated to adaptation were measured after 15 generations. Using a half-sib design, we investigated whether trait changes were related to genetic variation in the base population. Juvenile survival and fecundity exhibited genetic variation and increased in experimental populations on novel hosts. Conversely, no variation was detected for host choice and developmental time and these traits did not evolve. Longevity remained unchanged on novel hosts despite the presence of genetic variation, suggesting weak selection for this trait. Hence, patterns of evolutionary changes generally matched those of genetic variation, and changes in some traits were not hindered by long-term evolution in a constant environment.

Introduction

Adaptation to novel environments hinges on the interaction between a population and its environment, but also on the previous history of the population. Response to selection leading to adaptation occurs either through new mutations or through the genetic variation present in the original population (Orr, 2005). The smaller the founder population and the timeframe of the interaction between populations and environments, the more important the role of standing genetic variation in the process of adaptation (Hermisson & Pennings, 2005). It is generally believed that most traits exhibit significant genetic variation (Brakefield, 2003; Conner, 2003). However, long-term exposure to a homogeneous environment may lead to a loss of genetic variation, thus precluding adaptation to novel environments, at least within short timeframes (Dobzhansky, 1937; Falconer, 1989; Barton & Keightley, 2002). This may be due to a negative genetic correlation between alleles involved in adaptation to novel and to ancestral environments; fixation of a favourable allele in the ancestral environment would thus lead to the concomitant fixation of an allele that is disadvantageous in the novel environment, impeding adaptive change (Via & Lande, 1985; Via & Hawthorne, 2002). However appealing this hypothesis may be, such alleles have seldom been found (Via, 1990; Ballabeni & Rahier, 2000; Ueno *et al.*, 2003; Ahonen *et al.*, 2006; but see Hawthorne & Via, 2001). The genetic variation necessary to adapt to novel environments may be further exhausted after long periods of evolution in a constant environment because fixation is expected to occur at most loci (Bell, 1997; Barton & Keightley, 2002; Blows & Hoffmann, 2005; Barrett & Bell, 2006). Finally, adaptive evolution may be hindered by antagonistic pleiotropy among traits associated to adaptation to novel environments, even in the presence of genetic variation for these traits (Price & Langen, 1992; Lynch & Walsh, 1998).

Experimentally, adaptation to one environment has been shown to limit the ability to colonize other environments when evolutionary change relied solely

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on mutations (Buckling *et al.*, 2003). In experiments where standing genetic variation could play a role in adaptation, genetic variation for life-history traits in novel environments was sometimes lacking (Kawecki, 1995; Ueno *et al.*, 2003), whereas such variation was observed in other cases (Holloway *et al.*, 1990; Guntrip *et al.*, 1997; Hawthorne, 1997). In all these studies, organisms were either directly sampled from the field or only their recent evolutionary history was controlled for. Hawthorne (1997) showed that exposure to homogeneous environments (tomato or chrysanthemum plants) during 20 generations was not sufficient to result in a loss of genetic variation underlying adaptation to another environment (a leafminer-resistant chrysanthemum). Here, we use a population of spider mites reared on cucumber plants for more that 300 generations to test whether this prolonged period of evolution in a constant environment precluded the adaptation to novel environments. To test for the generality of our findings, we included two novel host plants, tomato and pepper and used five replicated populations for each host plant. Tomato and pepper are used by spider mites under natural conditions, and they are often heavily infested, indicating that there is sufficient genetic variance or phenotypic plasticity in spider mites to result in high performance on these plants. However, spider mites can form host races (Tsagkarakou *et al.*, 1997; Weeks *et al.*, 2000; but see Tsagkarakou *et al.*, 1998, 1999; Bailly *et al.*, 2004), suggesting that adaptation to one host could limit adaptation to other hosts.

Previous studies on experimental evolution using spider mites showed that mites quickly adapt to novel hosts (Gould, 1979; Fry, 1990; Agrawal, 2000). The populations used in those studies were sampled from many plants in the field, thus genetic variation for traits involved in adaptation is likely to have been present. In the spider mite population used in the present study, long-term evolution on cucumber might prevent further adaptation to tomato and pepper. To test this, we established experimental populations on tomato and pepper from the base population on cucumber. After at least 15 generations, we assessed the evolutionary response of mites from the experimental populations by measuring traits presumably involved in adaptation, namely juvenile survival, developmental time, adult longevity, fecundity and host choice. To assess how changes in trait values were related to the initial presence of standing genetic variation, we performed a quantitative genetic analysis of these traits in the base population.

The aims of our study were thus: (1) to test whether mites exposed to a single plant species during a long time period could still adapt to novel host plants, (2) to test how patterns of adaptation relate to the presence or absence of genetic variance in the base population and (3) to determine whether life-history trade-offs exist within each environment, possibly precluding adaptive evolution. If adaptation occurs in the experimental populations, then long-term evolution in a constant environment does not preclude adaptation to novel environments. This would indicate that strong trade-offs between adaptation to the ancestral and to each novel host were absent in the ancestral population at the onset of the experiment and that adaptation to the novel environments occurred through loci that were neutral in the ancestral environment.

Material and methods

Stock cultures

Plants were sowed once per week and cultured in a herbivore-free room under controlled conditions (25 °C). Cucumber seeds (variety Ventura) were provided by Rijkzwaan France, tomato seeds (variety Moneymaker) by Gebroeders Eveleens and pepper seeds (variety pikante reuzen) were obtained from the University of Wageningen.

Two-spotted spider mites (*Tetranychus urticae*, Koch) were reared in large numbers (> 10 000) on cucumber plants (approximately 4 weeks old, provided twice a week) under controlled conditions (25 °C) in a separate room. Spider mites were originally collected from a cucumber greenhouse (variety: Ventura) in Pijnacker, the Netherlands, in May 1994 and kept on that same variety in a climate chamber at the University of Amsterdam. The population at the University of Montpellier was established in April 2004 from approximately 10 000 individuals (all stages) sampled from the Amsterdam population. Calculating the number of generations encompassed by these 11 years of culture is approximate because the culture method is such that generations are overlapping, and life-history traits affecting generation time might have changed over time and with environmental conditions. We estimated the number of generations by using 13 days as the generation time, which is intermediate between 10 days from egg to egg, observed in Amsterdam and 15 days, observed in Montpellier (S. Magalhães, personal observation).

Experimental evolution and adaptation to novel hosts

All experiments were performed in an acclimatized room at approximately 25 °C.

Experimental populations on cucumber, tomato and pepper were established in March 2005 from the base population cultured on cucumber by placing 300 adult females on a detached leaf of each plant species. The petiole of each leaf was placed in a small vial (circa 5 cm diameter and 3 cm high) with water. The vial was covered with a small plastic lid with a few holes for the petioles and placed inside a plastic box (*c.* $20 \times 20 \times 10$ cm). The box was closed with a lid with a central hole covered with gauze and sealed with parafilm. It was placed in a tray containing water

with soap, to further isolate the experimental populations from one another. Leaves were added twice per week and they were discarded when they were too old (*c.* 10 days later). Five populations per host plant species were established. Each host plant represents a different selection regime. Traits were measured after all populations had been on the new host plants for approximately 15 generations. Owing to unforeseen problems, host choice was measured after *c.* 35 generations. Population sizes ranged from 100 to 1000 individuals in each box.

Evolutionary change was assessed by comparing life-history and behavioural traits on each novel host between populations evolving on the novel host and populations evolving on the ancestral host. Populations on the ancestral host were expected to reflect the ancestral state of the trait, corrected for changes due to environmental conditions during the experiment. Differences in trait values between mites evolving on the novel host or on the ancestral host provided an estimate of the change in the trait value due to adaptation to the novel host. All traits were measured on mites that had undergone one whole generation on bean, to minimize confounding effects of the individuals tested (or their mothers) having experienced different environments. Bean was chosen because mortality of mites from all experimental populations was negligible on this plant and fecundity was high and similar among experimental populations (data not shown). Hence, differential selection on each population during one generation on bean is highly unlikely. Specifically, females from each experimental population were allowed to oviposit on bean; these eggs developed to adulthood and the females that emerged laid eggs of their own on that same substrate. For measurements of juvenile survival, developmental time and fecundity and longevity, eggs were placed on each novel host plant as soon as they had been laid. For the host-choice experiment, the eggs laid were allowed to develop on bean, and the adult females that emerged were then tested.

Host choice was measured in adult female mites from each experimental population, by placing them on a tiny plastic roof between two flanking half-discs of different host plants (tomato and pepper, diameter 1.5 cm). Leaf discs were placed on water-soaked cotton wool in a plastic tray (20 × 10 × 3 cm; 6 double half-discs per tray). Their position was randomized to avoid biases caused by unforeseen directionality. Moreover, the position of the trays was shuffled regularly to minimize the possible effects of environmental heterogeneity. The number of eggs laid by each female on each plant type (tomato or pepper) was scored after 24 h. The number of females used per experimental population ranged from 10 to 15 (average: 12.7). During the choice period, females laid on average 1.6 eggs each (ranging between 1 and 4). The analysis was carried out on the proportion of eggs laid by each female on each leaf disc.

Assessment of juvenile life-history traits (juvenile survival and developmental time) was carried out by placing eggs from each experimental population on a tomato or a pepper leaf floating on water-soaked cotton wool inside plastic trays (20 × 10 × 5 cm). Survival was assessed approximately every 4 days during the first 12 days and every other day thereafter. Leaves were replaced approximately after 8 days because they became old. Several leaves of each plant were used for each population (3–7), one for each day on which eggs were laid and placed on the leaves. Individuals were counted either when they were dead, drowned in the water or when they became adults (male or female). We scored the age at which the event took place and the type of event. Sample size per experimental population ranged between 15 and 58 eggs (average: 29). Deutonymphs in the last moult received a male from the same experimental population, with which they could mate upon emergence. Developmental time was determined as the day that individuals reached adulthood. Daily fecundity (oviposition rate) was measured by placing mated females of each population on a leaf of the host plant on which they had developed and counting the eggs every 3 days during 12 days or until the female died. In this way, we obtained only one estimate of daily fecundity per experimental population. Leaves were replaced after each counting. The number of females used varied among populations and with time, ranging from 10 to 40 per population. In three out of five populations per selection regime, the experiment was prolonged until all females died, to assess total fecundity and longevity, except for cucumber populations on tomato, of which only two populations were tested.

Genetic variation in the base population

Genetic variation of all traits in the base population was assessed using a full-sib/half-sib design (Falconer, 1989; Lynch & Walsh, 1998). Males and female teleiochrysalids (the last resting stage before reaching adulthood) were collected from the stock culture and placed on a piece of cucumber leaf (approximately 1 cm^2) floating on water-soaked cotton wool inside a plastic container (diameter: 5 cm). Each male was allowed to mate with eight females. As soon as the females had mated, they were isolated on a piece of cucumber leaf to produce full-sib families. The offspring of all females that had mated with the same male (sire) were either full-sib (same mother; dam) or half-sib (different mothers). Juvenile traits, host choice and adult traits were measured in independent experiments resulting from different crosses.

To assess host choice, the offspring of each cross was allowed to develop on cucumber. As soon as they reached maturity, females mated to their brothers, and they were subsequently tested. Spider mites are arrhenotokous parthenogenetic, thus inbreeding depression resulting from sib-mating is unlikely (Henter,

2003). Because some F0 and F1 females were unmated or eliminated for other reasons, and the remaining females did not produce the same number of offspring, the design was unbalanced for all traits. Host choice was measured in the F1 females as described for the adaptation trials. The experiment was performed in four blocks spaced between January and August 2005. A total of 959 F1 females were tested, produced by 28 sires and 139 dams.

Developmental time and juvenile survival were measured by placing eggs from each full-sib family on either pepper or tomato leaf discs. These traits were measured as described for the adaptation trials, except that they were grouped by three instead of placing all eggs of a population on the same leaf. The experiment was repeated in two blocks (February–March 2005 and July–August 2005). Juvenile survival was assessed in 1354 individuals (696 on pepper and 648 on tomato respectively), produced by 18 sire and 104 dams. Developmental time was assessed in 281 individuals (199 on pepper and 82 on tomato respectively), coming from 17 sires and 86 dams, because only individuals that had reached maturity were used (other individuals either died before reaching adulthood or they drowned in the water).

To measure fecundity and longevity on each host, eggs were let to develop on cucumber, and mated F1 females were placed on either a tomato or a pepper disc. The eggs that they produced were counted approximately every other day until the females died. The date of death was recorded to estimate longevity (note that developmental time is not included in this measure). Females that drowned in the water during the experiment were discarded from the analysis ($n = 71$). There were four blocks, 20 sires, 226 dams and 3415 individuals tested (1705 on pepper and 1710 on tomato respectively).

Statistical analysis

Experimental evolution and adaptation to novel hosts

Differences in life-history traits among selection regimes were tested by comparing experimental populations on each host plant species with a GLM procedure in SAS with selection regime as a factor. For developmental time, longevity and host choice we included a random factor of replicate population within selection regime and tested effects of the selection regime against this factor. Because individual data were collected for these three traits, we could also test for differences among replicate populations within each selection regime. For developmental time, gender was included as an additional factor, as well as its interactions with all the other factors. The analysis confirmed that males develop significantly faster than females, impeding the merging of the two genders in one factor. However, the inclusion of gender in the analysis did not modify the main results, probably because the number of males that reached adulthood was very low, due to a female-biased sex ratio and high juvenile mortality in both sexes. Therefore, we opted for performing the analysis on the females only (this is valid for the analysis of the half-sib design as well). Differences in survival on each host plant were tested using the LIFETEST procedure using SAS with a Log-rank test. Individuals that reached maturity (males and females) or that drowned in the water during the experiment were coded as censored, whereas individuals that died were uncensored.

Data on host choice was arcsin-square root transformed and data on developmental time on tomato were exponentially transformed, to comply with ANOVA assumptions. The occurrence of phenotypic correlations among changes in life-history traits was tested using the Pearson correlation for all traits in all experimental populations and host plants tested.

Genetic variation in the base population

To test whether additive genetic variance (differences among sires) and maternal effects and/or dominance effects (differences among dams) for host choice were significant, we used a GLM procedure in SAS with replicate, sire and dam as random factors (sire nested within replicate, dam nested within both).

Juvenile survival was analysed by a log-rank test using the PHREG procedure in SAS (the multi-factorial equivalent of the LIFETEST procedure), with replicate, sire, dam and plant as factors. Sire was nested in replicate, and dam was nested in sire and replicate; the interaction between plant and all these factors was tested as well.

Differences in developmental time, fecundity and longevity were tested using the GLM procedure in SAS with replicate, sire, dam and plant as factors. Sire was nested within replicate, and dam was nested within sire and replicate; these three factors were treated as random. Plant was a fixed factor and the interactions between plant and the other factors were treated as random (Sokal & Rohlf, 1995). If the interaction between plant and the other factors was significant, the factors included in the interaction were tested against the interaction term. If it was not, the interaction term was included in the error term (Sokal & Rohlf, 1995).

To obtain the variance components for each trait in each environment, separate ANOVAS were performed for the data on each plant. Survival analysis does not allow for the calculation of variance components. Therefore, we calculated mortality per dam and did an ANOVA on this fraction, with block and sire nested within block as factors. The variable was arcsin-square root transformed to comply with normality. Variance components were estimated using the VARCOMP procedure in SAS with Restricted Maximum Likelihood (REML) estimates because the data set was unbalanced (Lynch & Walsh, 1998).

Additive genetic variance (V_a) in the base population was calculated from the sire variance component (σ_S) using the formula for haploid organisms $\sigma_S = 1/2V_a$ (Olson & Andow, 2002). Subsequently, we calculated the heritability (V_a/V_p; V_p = phenotypic variance) and evolvability ($\sqrt{V_a}/X$; X = trait mean) for each trait. To estimate the confidence intervals of V_a, we generated 1000 simulated data sets by resampling sires, then recalculated V_a using the procedure described above. To calculate $V_m + V_d$ and trait means, simulated data sets were generated by resampling dams and individuals respectively. Confidence intervals for all parameters were subsequently estimated as the interval comprising 95% of the values obtained for each trait.

The genetic covariance between fecundity and longevity within each environment was calculated following the method described in Messina & Fry (2003) and Fry (2004). Using the MIXED procedure of SAS, this method tests the significance of covariances among traits within each level of variance partitioning (block, sire, dam and individual). Based on the covariances obtained, the genetic correlation was calculated (Sokal & Rohlf, 1995).

Results

Experimental evolution and adaptation to novel hosts

Host choice was not significantly different among selection regimes (Fig. 1; $F_{2,5.03} = 1.19$; $P = 0.38$, average proportion of cucumber populations choosing tomato: 0.7, of pepper populations: 0.67, of tomato populations: 0.53). No significant difference was found among experimental populations (Fig. 1; $F_{5,91} = 1.13$; $P = 0.35$).

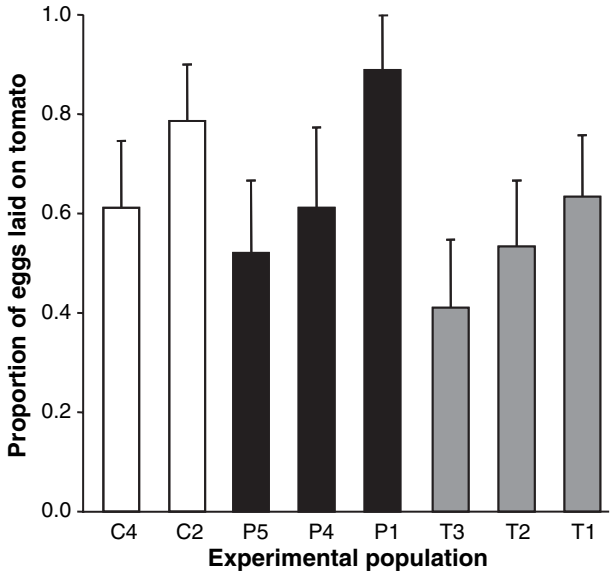

Fig. 1 Host choice of female spider mites from each experimental population (indicated below the *X*-axis). The graph shows the proportion of eggs laid on tomato during 24 h. White bars correspond to populations from cucumber, black bars to populations from pepper and grey bars to populations from tomato. Mites from each population had undergone one generation on bean before being tested. Vertical lines correspond to the standard error of the mean.

On pepper, mites from experimental populations on pepper had higher juvenile survival than mites from populations on cucumber (Fig. 2a–b; Log-Rank test, effect of selection regime, $\chi^2_1 = 3.88$, $P = 0.046$). Similarly, mites from the tomato selection regime showed higher juvenile survival on tomato than mites from the cucumber selection regime (Fig. 2c–d, Log-Rank test, effect of selection regime, $\chi^2_1 = 32.66$, $P < 0.0001$). Hence, evolution on the novel host plants led to an adaptive change in juvenile survival on both plants. On pepper, differences among populations within selection regimes were significant among cucumber populations but not among pepper populations (Log-rank test, $\chi^2_1 = 16.93$ $P = 0.002$ and $\chi^2_1 = 4.65$ $P = 0.33$ respectively). Conversely, on tomato, heterogeneity was not found among cucumber populations whereas it was significant among tomato populations (Log-rank test, $\chi^2_1 = 0.92$ $P = 0.92$ and $\chi^2_1 = 36.89$ $P < 0.0001$ respectively).

On pepper, developmental time of mites from the pepper populations did not differ from that of mites from the cucumber populations (Fig. 3a, GLM, effect of selection regime, $F_{1,17.36} = 0$, $P = 0.95$, average of cucumber populations: 20.44 ± 1.19, of pepper populations: 20.5 ± 0.69). Similarly, developmental time on tomato did not differ among selection regimes (Fig. 3b, GLM, effect of selection regime, $F_{1,7.09} = 1.01$, $P = 0.35$, average of cucumber populations: 19.39 ± 1.19, of tomato populations: 17.23 ± 1.44). Therefore, the environment experienced by populations during 15 generations did not affect their developmental time. On pepper, differences among populations within each selection regime were significant for pepper populations but not for cucumber populations ($F_{4,51} = 2.63$, $P = 0.044$ and $F_{3,39} = 1.21$, $P = 0.31$ respectively). On tomato, these differences were even more conspicuous ($F_{4,36} = 130.8$ $P < 0.0001$ and $F_{3,64} = 14.2$, $P < 0.0001$, among tomato and cucumber populations respectively).

On pepper, daily fecundity of the pepper populations was significantly higher than that of the cucumber populations (Fig. 4a, GLM, effect selection regime, $F_{1,8} = 14.52$, $P = 0.0052$; mean of cucumber populations: 0.24 ± 0.07, of pepper populations: 0.95 ± 0.17). Similarly, on tomato, daily fecundity of mites from the tomato populations was significantly higher than that of mites from the cucumber populations (Fig. 4b, GLM, effect of selection regime, $F_{1,7} = 9.6$, $P = 0.017$; mean of cucumber populations: 0.47 ± 0.06, of tomato populations: 1.16 ± 0.24). This indicates that populations on each novel host were selected for higher daily fecundity on that host.

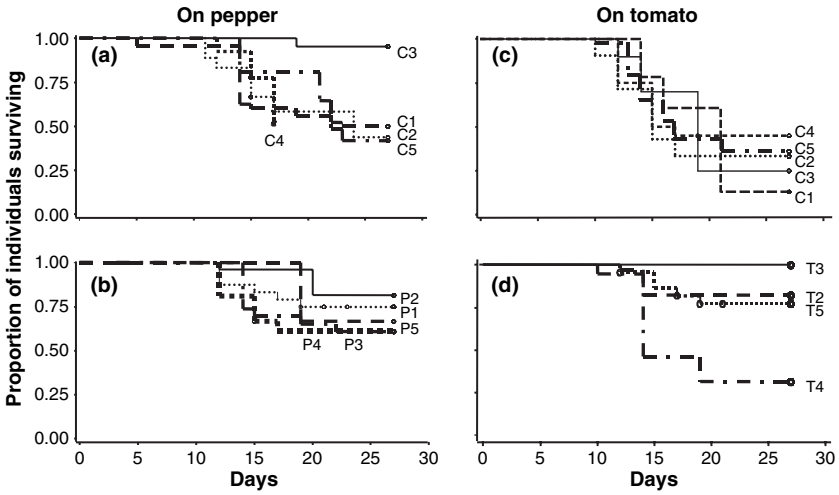

Fig. 2 Juvenile survival of mites from different experimental populations (a, c: from cucumber; b: from pepper; d: from tomato) on two new host plants (a–b on pepper; c–d: on tomato). The graph shows the proportion of individuals surviving in each population. The last data point corresponds to the proportion of individuals that survived to adulthood. Circles indicate censored individuals.

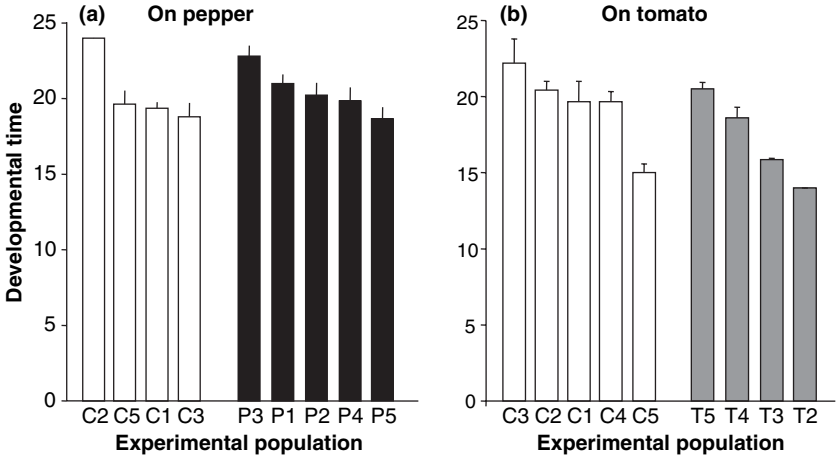

Fig. 3 Developmental time of experimental populations (white bars = from cucumber; black bars = from pepper; grey bars = from tomato) on pepper (a), tomato (b). Each bar corresponds to one experimental population, indicated along the *X*-axis. Error bars correspond to standard errors of the mean.

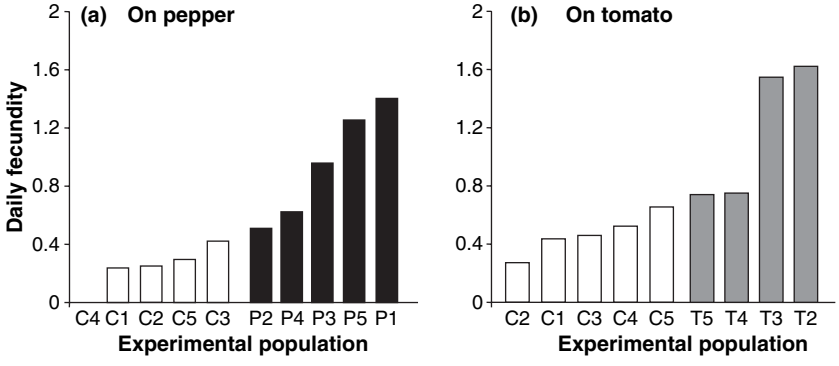

Fig. 4 Daily fecundity (number of eggs per female per day) of mites from experimental populations (white bars = from cucumber; black bars = from pepper; grey bars = from tomato) on pepper (a), or tomato (b). Each bar corresponds to one experimental population, indicated along the *X*-axis.

Longevity on the novel host plants was not affected by the selection regime (Fig. 5a,b, GLM $F_{1,4.29} = 0.04$, $P = 0.85$, $F_{1,3.02} = 1.01$, $P = 0.39$, on pepper and on tomato respectively). On pepper, populations within selection regimes did not differ in longevity ($F_{2,20} = 1.9$, $P = 0.17$, average: 34. 89 ± 1.6 for cucumber populations and $F_{2,20} = 1.03$, $P = 0.37$, average: 35.22 ± 2.01 for pepper populations respectively). On tomato, there was significant variation in longevity among populations from cucumber, whereas no difference was found among

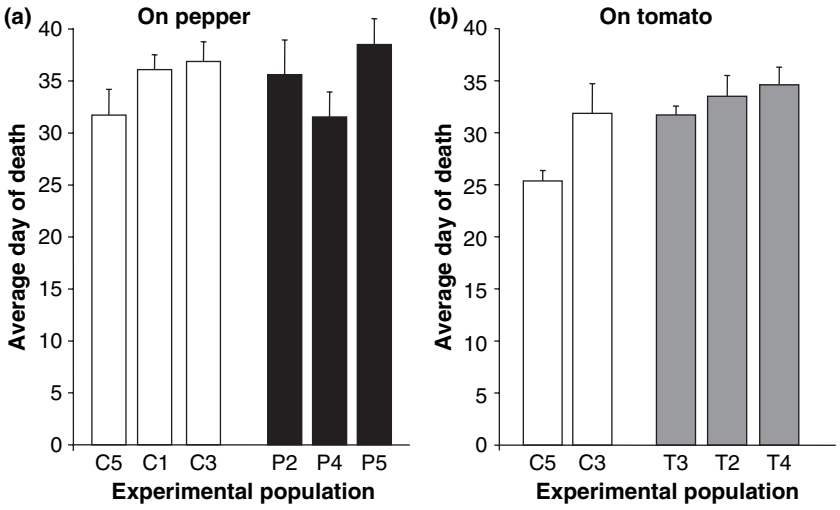

Fig. 5 Longevity of individuals from experimental populations (white bars = from cucumber; black bars = from pepper; grey bars = from tomato) on pepper (a) and tomato (b). Each bar corresponds to one experimental population, indicated along the *X*-axis. Error bars indicate the standard error of the mean.

Table 1 Correlations among life-history traits in all populations.

	Fecundity	Longevity	Developmental time	Juvenile mortality
Fecundity	1	0.09	− 0.56	− 0.73
		0.79	*0.015*	*0.0004*
		11	18	19
Longevity		1	–0.34	0.37
			0.29	*0.25*
			11	11
Developmental time			1	0.37
				0.12
				18

Given are the Pearson correlation coefficients followed by their corresponding *P*-value (in italic) and by the sample size for each correlation.

Table 2 ANOVA table for host choice.

Source	d.f.	*F*	*P*
Block	3	25.95	< 0.0001
Sire (block)	24	0.86	0.6478
Dam (sire block)	111	1.13	0.1808

tomato populations (respectively $F_{1,28} = 6.97$, $P = 0.014$, average: 28.6 ± 3.01, and $F_{2,42} = 0.95$, $P = 0.39$, average: 33.27 ± 0.84).

Taking all populations in all environments into account, we found a significant negative correlation between daily fecundity on the one hand and developmental time and juvenile mortality on the other hand (Table 1). This indicates that the populations in which individuals had higher daily fecundity were also the ones in which juveniles survived better and developed faster. Developmental time was not correlated to juvenile survival, and longevity was not correlated to any trait.

Genetic variance in the base population

A significant effect of the block and/or of the interaction between block and plant was observed for all traits except fecundity (Tables 2–4). This indicates that there was significant variation in trait values among blocks (i.e. in time) in either host-plant quality or in the mites from the base population.

A significant sire effect was found for juvenile survival, fecundity and longevity, but not for host choice and developmental time (Tables 2 and 3). A significant dam effect was found for juvenile survival, but not for any other trait. Genotype-by-environment interactions were observed for juvenile survival only (the sire × plant interaction was significant; Table 3). The interaction between dams and plants was significant for juvenile survival and fecundity only (Table 3).

Within each selection regime, there was a significant sire effect on survival, fecundity and longevity on each plant (Table 4), confirming the significant effect found in the main analysis and indicating that there is additive genetic variance for these traits. Evidence for additive genetic variance in developmental time was not found. The dam variance component was significant on both plants for juvenile survival and developmental time (Table 4). For fecundity and longevity, the dam component was significant on tomato only. Thus, adaptation can be further fostered by dominance and/or maternal effects, especially on tomato. As no significance was found for dam or dam × plant factors for developmental time and longevity in the previous analysis, results on these traits are probably due to a different partitioning of the variance in the two analyses.

Based on the GLMS performed within each plant species, we calculated the additive genetic variance,

Table 3 Results of the analyses of life-history traits on both plants.

	Juvenile survival		Developmental time		Fecundity		Longevity	
Source	χ^2	*P*	*F*;d.f.	*P*	*F*;d.f.	*P*	*F*;d.f.	*P*
Block	52.2	< 0.0001	81.52;1	< 0.0001	0.35;3	0.79	3.2;3	< 0.0001
Sire (block)	26.17	0.0008	1.52;15	0.12	3.53;16	< 0.0001	2.52;16	0.003
Dam (sire block)	90.88	< 0.0001	1.07;86	0.48	0.94;95	0.63	1.23;95	0.16
Plant	9.95	0.0016	1.57;1	0.43	0.54;1	0.51	16.37;1	0.023
Plant × Block	20.42	< 0.0001	0.32;1	0.58	10.74;1	0.0004	9.16;1	0.0009
Plant × sire (block)	34.7	< 0.0001	0.51;14	0.89	1.01;16	0.65	0.45;16	0.97
Plant × dam (sire block)	77.06	< 0.0001	1.54;14	0.11	1.44;91	0.005	1.05;91	0.35

Table 4 Analysis of traits within each plant.

		Juvenile survival		Developmental time		Fecundity		Longevity	
Plant	Source	χ^2	*P*	*F*;d.f.	*P*	*F*;d.f.	*P*	*F*;d.f.	*P*
Pepper	Block	64.19	< 0.0001	74.29;1	< 0.0001	2.99;3	0.06	4.89;3	0.013
	Sire (block)	8.04	0.005	1.08;15	0.4	1.99;16	0.022	1.89;16	0.031
	Dam (sire block)	100.46	< 0.0001	1.55;54	0.04	1.12;93	0.21	1.07;93	0.31
Tomato	Block	17.22	< 0.0001	90.02;1	< 0.0001	2.16;3	0.13	1.48;3	0.26
	Sire (block)	26.335	< 0.0001	1.36;16	0.23	2.43;16	0.004	1.76;16	0.046
	Dam (sire block)	66.32	< 0.0001	3.14;25	0.003	1.6;93	0.0006	1.38;89	0.01

Table 5 Trait means, variance components, heritabilities and evolvabilities.

Trait	Plant	Trait mean	V_a	$V_d + V_m$	h^2	CV_a
Fecundity	Tomato	10.24 (1.12)	14.75 (11.9)	9.34 (7.2)	0.11 (0.07)	0.38 (0.18)
	Pepper	8.52 (0.27)	5.19 (3.51)	1.70 (0.91)	0.06 (0.012)	0.27 (0.05)
Longevity	Tomato	7.42 (0.39)	0.40 (0.21)	0.36 (0.7)	0.04 (0.023)	0.09 (0.037)
	Pepper	9.22 (0.6)	0.73 (0.34)	0.22 (0.8)	0.05 (0.034)	0.09 (0.054)

Only values for the traits yielding a significant sire component in the ANOVA within each plant are given. Longevity was measured from the moment that females became adult until they died. Confidence intervals are given between brackets.
V_a, additive genetic variance; V_d, dominance variance; V_m, variance due to maternal effects; h^2, heritability; CV_a, additive genetics coefficient of variation (evolvability).

dominance variance, heritability and coefficient of genetic variance (evolvability) for the variables that yielded a significant sire component (Table 5). Although the analysis of juvenile survival indicated that all effects and their interactions were significant (Table 4), the sire component was not significant in the ANOVA performed subsequently to extract the variance components (on pepper $F_{16,81} = 1.42$ $P = 0.16$; on tomato $F_{16,84} = 0.94$ $P = 0.53$). Probably, this is because of a lack of statistical power of the latter analysis caused by the need to analyse differences among proportions surviving in each dam within one sire, instead of analysing differences among survival curves. Fecundity had higher additive genetic variance and dominance and maternal variance than longevity (Table 5). Concomitantly, its heritability and coefficient of genetic variance were higher. This difference was more conspicuous for fecundity on tomato than for fecundity on pepper. Heritability and the coefficient of genetic variance gave similar results in the ranking order of variables and these values were generally low.

Overall, individuals tended to have higher longevity and lower fecundity on pepper than on tomato (Table 5), suggesting a negative environmental correlation at the 'macro-environment' level. To investigate whether this correlation could be explained by a physiological trade-off between these traits, we measured the genetic correlation between these traits in each environment. This genetic correlation was not significant (Table 6). In addition, there was a strong positive environmental correlation at the micro-environment level: on both plants, individuals that laid more eggs also survived longer (Table 6). A significant positive environmental correlation was also found between longevity and daily fecundity of the first 10 days of oviposition (data not shown). This indicates that the positive environmental correlation between fecundity and survival is not a

Table 6 Environmental and genetic correlations between longevity and fecundity, followed by their correspondent *P*-value.

Plant	Component	Value	*P*
Tomato	Genetic	1.12	0.16
	Maternal	0.81	0.16
	Environmental	0.75	< 0.0001
Pepper	Genetic	0.17	0.81
	Maternal	0.44	0.76
	Environmental	0.69	< 0.0001

simple consequence of long-lived mites laying eggs for a longer period.

Discussion

Mites from a population established on cucumber for more than 300 generations and evolving on novel hosts during 15 generations showed changes in some traits associated to adaptation to those hosts. Because adaptation occurred within a relatively short time span and the population sizes in the experimental populations were not extremely large (between 100 and 1000 individuals), the responses observed were probably caused by the genetic variance present in the original population, rather that by the occurrence of new mutations. Hence, some genetic variation for traits associated to adaptation to novel hosts was maintained after many generations of evolution on a single ancestral host species and underlied adaptation to novel hosts.

Adaptation was detected in experimental populations on each novel host plant, because on average populations evolving on the novel hosts had higher trait values than populations on the ancestral host. However, there was a considerable amount of variation among populations, both for populations from the ancestral host and for those from the novel hosts. For example, juvenile survival of mites from the cucumber populations on pepper ranged from zero to nearly 100%. This may be a result of environmental variation while measuring trait values, but variation was not detected in all traits or in all selection regimes, suggesting that there are inherent differences among the degrees of variation of traits. Because each experimental population represents only a subset of the variation occurring in the base population, this sampling may have introduced a bias in some traits. In populations evolving on novel host plants, it is also possible that a strong bottleneck in the first generations, as a result of high mortality, increased the variation in the responses among populations. In general, it is rather conspicuous that adaptation is not very replicable among populations. For example, two tomato populations (T2 and T3) had very high daily fecundity, whereas two other populations had trait values similar to those of cucumber populations, suggesting little evolution of this trait for those populations. This is not correlated with the initial number of spider mites present in the populations, as all populations were started with 300 females and did not show conspicuous differences in the population size thereafter (data not shown; note that effective population sizes are unknown). The variability in the evolutionary changes observed can be seen as a peculiarity of our experiment, because the initial number of spider mites was not very high and the selection intensity possibly very strong, thus limiting the available variability. However, such variation in responses is found in many studies on experimental evolution and even when initial numbers are very high (Lenski *et al.*, 1991; Hawthorne, 1997; Sniegowski *et al.*, 1997; Wichman *et al.*, 1999; Teotonio & Rose, 2000; Bochdanovits & de Jong, 2003; Grimberg & Zeyl, 2005; Kawecki & Mery, 2006; Woods *et al.*, 2006 but see Pelosi *et al.*, 2006), suggesting that variability in the adaptation process could be a general feature. Hence, more experimental evolution studies in which the evolutionary process is replicated are needed.

Our quantitative genetic analysis of the base population confirmed the occurrence of genetic variation in the traits that have subsequently evolved, namely fecundity and juvenile survival. Conversely, no genetic variance was detected for host choice and developmental time, and these traits did not evolve. As our novel environments were homogeneous with respect to host plant, host choice was not expected to evolve even if we had found genetic variation for this trait. In contrast, developmental time was expected to be under strong selection, since it determines the onset of the reproductive period, which strongly affects the intrinsic growth rate in mites (e.g. Janssen & Sabelis, 1992; Magalhães *et al.*, 2003). Therefore the lack of response in this trait is probably a result of the lack of genetic variation found, even though the heterogeneity found in this trait among experimental populations on cucumber suggests that there is some variation for this trait in the base population.

Genetic variability in the base population was thus generally a good predictor of genetic change due to selection. However, this was not the case for all traits. Indeed, genetic variance for longevity was detected but this trait did not evolve. In the set-up used for the experimental populations, resources were continuously renewed and rarely overexploited Therefore, density dependence was not expected to operate in these populations. Under those circumstances, the intrinsic growth rate is expected to be a better proxy for fitness than life-time reproductive success (R_0) (Mylius & Diekmann, 1995; Brommer, 2000), and, as the population was growing, longevity was probably not under strong selection. Because this trait was not correlated to the traits that were under selection (juvenile survival, developmental time and early fecundity), it was not expected to evolve in the new environment, despite being genetically variable in the ancestral population. Quantitative genetics designs have been often been used

to generate predictions on artificial selection experiments, usually with successful results (Etges, 1998; Hansen & Shrestha, 1999; Juenger & Bergelson, 2000; Delph *et al.*, 2004, but see Czesak *et al.*, 2006). In experimental evolution, the selection coefficients of traits are not known *a priori*. Thus, whenever no genetic variation and no evolutionary change are found, one can not distinguish between a lack of selection pressure and a lack of variation for the trait. However, when mismatches between genetic variation and evolutionary response occur, quantitative genetic analyses may contribute to the understanding of the patterns observed (e.g. Milanovic & Gliksman, 2004), as is the case with longevity in our experimental populations.

Genetic variability in some traits was detected despite a considerable amount of environmental variability, as shown by the significant block effect in the half-sib analysis. Hence, although we meticulously attempted to maintain conditions constant (same plant variety, constant controlled conditions in the culture room and in the laboratory, randomizing positions in the experimental trays, etc.), there was still a considerable amount of residual environmental variability. This variability may stem from temporal variation in plant quality or from mites of the base population exhibiting different responses through time (e.g. seasonal effects). Therefore, the concept of homogeneity in the environment (or in the interaction between a single population and a single environment) should be handled with caution. In agreement with this, it is known that single populations may vary in their preference between two resources or in their performance on a single resource, depending on the individual resource offered or on temporal factors (Singer & Lee, 2000; Cronin *et al.*, 2001; Garant *et al.*, 2004). Such variability was also found in spider mites (Wilson, 1994; Krips *et al.*, 1998). This environmental variability may also contribute to the maintenance of genetic variation in the base population of this study.

The lack of a negative genetic correlation between longevity and daily fecundity in the base population suggests the absence of a trade-off between these traits. However, a trade-off could exist, but be masked by other sources of variation (e.g. environmental or genetic variation for the acquisition of resources) being several orders of magnitude higher than genetic variation among these traits. The positive environmental correlation between longevity and fecundity at the individual level suggests that there are some micro-environmental differences in the amount of resources acquired by individuals, and that such differences do not affect how these resources are allocated to various fitness components. At the macro-environmental level (between plants), mites tend to have higher fecundity and lower longevity on tomato than on pepper. This negative environmental relationship in longevity and fecundity between host plants suggests that different plants induce a different pattern of resource allocation between survival and reproduction in mites. Alternatively, it could be that tomato is a favourable host for fecundity and pepper for longevity.

In the experimentally evolving populations, a negative phenotypic correlation between changes in fecundity, developmental time and juvenile mortality across populations was found. That is, populations with higher fecundity also exhibited lower juvenile mortality and shorter developmental time. This suggests that selection for the different life-history traits has proceeded in parallel and that the trade-off curve, in which resources are differentially allocated to each trait, has not been reached (van Noordwijk & De Jong, 1986; Delaguerie *et al.*, 1991; Houle, 1991). Moreover, genotype-by-environment interactions were not detected in the base population. Therefore, trade-offs between adaptations to the two novel hosts are not expected at this stage.

The lack of trade-off between adaptation to different hosts is in agreement with the other studies on experimental evolution with spider mites (Gould, 1979; Fry, 1990; Agrawal, 2000). Indeed, Gould (1979) found that mites from populations selected on cucumber did not reduce their performance on their original host, bean, after approximately 50 generations. In addition, he found that selection on cucumber had a positive effect on juvenile survival on other hosts. Fry (1990) as well as Agrawal (2000) found that selection on the novel host did not entail reduced performance on the ancestral host. Moreover, they both found that mites from lines that were first selected on a novel host and then returned to the ancestral host for several generations did not show reduced performance on the novel host, relative to the performance of mites at the time that the reversion took place. This indicates that selection on the ancestral host in these reverted lines did not affect their performance on the novel host. In our study, a population occurring on cucumber during 300 generations could still adapt to novel host plants, suggesting a lack of antagonistic pleiotropy between loci involved in adaptation to different hosts. As cucumber is an annual crop, spider mites are not expected to occur for such a long time period on that host under natural conditions. As even in our experiments adaptation to novel hosts plants was not hindered by long term adaptation on cucumber, it seems unlikely that it will be the case under natural conditions. Taken together, these results suggest that trade-offs are not limiting the host range of spider mites. Therefore, the occurrence of host races (Tsagkarakou *et al.*, 1997; Weeks *et al.*, 2000) calls for an ecological explanation.

In summary, adaptation to different host plants in spider mites does not seem to be hindered by trade-offs or by lack of genetic variability, at least within the time frame of our experiments. Therefore, our results do not support the hypothesis that the host range of organisms is limited by specialization on one host. Overall, it can be tentatively concluded that selection on one host does not preclude evolutionary change on other hosts. This raises

(or leaves open) the question of whether there are intrinsic limits to host ranges.

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