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Range expansion induces variation in a behavioural trait in an ant–plant mutualism

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Climate oscillations produce dramatic changes in species distribution, even in the tropics. The ant–plant Leonardoxa africana africana hosts and feeds the ant Petalomyrmex phylax, which provides protection against herbivores in return. Both partners of this symbiosis present a recent southward range expansion. To test whether the higher investment in sexuals (and thus lower investment in protective workers) previously documented on the colonization front is compensated by a more effective protective behaviour, we compared ant behavioural investment in plant defence between two populations, one in the core of the range and one on the colonization front. We induced ant patrolling activity by artificially damaging leaflets and measured this activity by counting patrolling ants and calculating the increase relative to constitutive patrolling activity measured on control (undamaged) leaflets. Contrary to our expectation, ant behavioural investment in plant defence was lower on the colonization front. Thus, production of fewer workers is not compensated by more protective behaviour of each. Instead, both traits contribute to a phenotype that is less mutualistic as a whole. By favouring increased allocation to dispersal, range expansion can shape ant behavioural traits and potentially the outcome of mutualism.

1. Introduction

Periodic variation in parameters of the Earth’s orbit provokes large climatic variations, called Milankovitch oscillations, at time scales ranging from tens of thousands of years to over 100,000 years (Berger, 1988). Variations in temperature and precipitation produce dramatic changes in species distributions. Such “orbitally forced species range dynamics” (ORD) (Dynesius and Jansson, 2000; Webb and Bartlein, 1992) are considered a major driver of evolution, shaping life history traits, interspecies interactions, and global patterns of biodiversity at different scales (Dynesius and Jansson, 2000). The amplitude of Milankovitch oscillations is positively correlated with latitude, and the resulting ORD is much more pronounced in temperate and boreal environments of the Northern hemisphere than in the tropics. This could help explain latitudinal gradients in species diversity and in the sizes of species’ geographical ranges. Generalists with high dispersability are thus selected (both within populations and among lineages) at high latitudes, whereas near the equator low ORD permits the persistence of specialist species with narrow ranges (Dynesius and Jansson, 2000). However, while climatic fluctuations are less pronounced in the tropics, they still result in contraction/expansion cycles of species ranges. During range expansion, phenotypes with higher dispersal capacities are expected to dominate at colonization fronts (Excoffier et al., 2009; Travis and Dytham, 2002). This phenomenon has been documented in various organisms and environments (Léotard et al., 2009; Phillips et al., 2006; Thomas et al., 2001). The pace of evolutionary processes differs among organisms according to generation time. Selection at colonization fronts should thus lead to evolutionary increase in dispersal capacity more rapidly in species with shorter generation times.

The stability of mutualistic symbiotic interactions depends on a good match between the interests of the partners. Therefore, in symbioses involving organisms with generation times that differ by orders of magnitude, selection at colonization fronts during range expansion could generate conflicts between mutualists, owing to shifts in the costs and benefits for each partner. Moreover, when selection favours higher dispersal capacity this can affect other life history traits that influence the costs and benefits for partners.

In most ant–plant symbioses, plants provide symbiotic ants with nesting cavities (specialized hollow structures called domatia) and food (extra-floral nectar and/or food bodies). In return, ants
protect their host plants against herbivores, pathogens, and competing plants (Letourneau, 1998; Rosumek et al., 2009; Suarez et al., 1998). These mutualisms are horizontally transmitted, i.e., the association must be re-established each generation, each partner dispersing independently of the other. Thus, each partner gains a benefit from the growth of the other partner, but not from its reproduction. Only when dispersal of both ant and plant is very limited is there a high probability that foundresses from a particular colony will colonize progeny of their natal tree (Szilágyi et al., 2009). The reproductive allocation of one partner thus usually does not benefit the other. Furthermore, reproduction and growth draw from the same pool of limited resources. For each partner, the greater its investment in reproduction, the lower its allocation to environmental niche partitioning (Debout et al., 2009).

The tree *Leonardoxa africana* subsp. *africana*, hereafter *L. africana*, is associated with the mutualistic ant *Petalomyrmex phylax*, which lives in its hollow internodes and feeds on its foliar nectar (McKey, 1984). This ant–plant symbiosis is endemic to coastal equatorial rainforests in Cameroon (McKey, 2000). Workers constitutively patrol the young leaves of the host plant day and night (Gaume and McKey, 1999); workers also patrol mature leaves when they are damaged, responding to volatile organic compounds they emit (Brouat et al., 2000; Schatz et al., 2009). Patrolling effectively protects the plant against herbivores (Gaume et al., 1997). The ant *Catalaulus mckeyi* also nests only in hollow internodes of *L. africana*. This ant is considered a parasite of the mutualism because it takes advantage of the myrmecophytic features of the plant without protecting it (Gaume and McKey, 1999) and prevents or delays occupation by the mutualist. The coexistence of this parasite (usually at lower densities) and the mutualistic ant is explained by a combination of trade-offs related to environmental niche partitioning (Debout et al., 2009).

A recent study focused on the effect of range expansion on dispersal evolution in this three-species symbiosis (Léotard et al., 2009). The three species present congruent genetic signatures of recent gradual southward expansion. Further, in agreement with theoretical predictions, both ant species present a more dispersive strategy close to the colonization front. A whole suite of traits characterize this more dispersive strategy (Dalecky et al., 2007; Léotard et al., 2009). First, similar-sized colonies produce more sexual females and fewer workers at the southern front than in the median part of the distribution. Moreover, sexual females of *P. phylax* from the colonization front are larger and survive longer without food, increasing both their dispersal capacity and their success in claustral colony foundation. These colony characteristics should influence mutualistic benefits, because reduction in the proportional investment in workers could result in less effective defence of the plant.

In contrast, the host plant showed no difference in tree size at first reproduction, a trait that should be linked to dispersal capacity. Moreover, no difference was detected in the number of foliar nectaries, indicating that the plant has not reduced provision of resources to ants at the colonization front, as might be expected if selection favours “sanctions” against less effective mutualists (Bull and Rice, 1991).

Effectiveness of the protection provided by ants depends not only on the number of workers produced relative to alates, but also on worker aggressive behaviour towards herbivores. We tested the hypothesis that individual workers of the mutualistic ant *P. phylax* have a more effective protective behaviour at the colonization front, compensating for the reduced proportional investment in workers, and thus explaining the maintenance of mutualistic traits in the plant.

### 2. Materials and methods

We performed behavioural assays in the same two populations of *P. phylax* that Léotard et al. (2009) and Dalecky et al. (2007) used for comparing life-history traits: N’kolobondé (BON, 3°13’18” N, 10°14’57” E), in the median part of the distribution, and Ebodjé (EBO, 2°34’05” N, 9°50’37” E), at the southernmost edge of the range (Fig. 1). Twenty colonies were studied in each population. We tested ant attraction to experimentally damaged leaflets, simulating herbivory, using a standard paired-leaf design (Agrawal, 1998; Lapola et al., 2003; Romero and Izzo, 2004). *L. africana* has paripinnately compound leaves with opposite leaflets. For trials, we used one pair of basal leaflets per colony, and thus per tree. For each leaflet pair, control and experimental treatments were assigned randomly. Tests were performed during the period of ant activity (11:00–15:00), and we chose leaves on which ants were active. ‘Experimental’ leaflets were damaged by cutting a triangular piece from the margin of the lamina. ‘Control’ leaflets were only touched with the scissors. Workers of *P. phylax* quickly retreat into the domatia when their plant is handled by an experimenter (McKey, 1984). Manipulation of leaflets in our experiment usually resulted in all the ants leaving those leaflets. The lag time between manipulation of a leaflet and its contact by a new ant was recorded. As soon as a new ant contacted one of the focal leaflets, we started to count the number of ants on each leaflet every minute for 20 min. First, for each test we computed the mean (over the 20 min) of the number of ants on the experimental leaflet, and on the control leaflet. For each test we also computed the mean of the difference in numbers of ants between the experimental and control leaflets. For each population we tested whether the number of ants on experimental leaflets was higher than on control leaflets, to test for the effect of experimental damage on ant attraction. We then compared these values between the two populations BON and EBO. Because ant colonies of these two populations differed in their investment in workers (Léotard et al., 2009), we may expect a difference in the number of ants on focal leaflets that could merely be a consequence of differences in total number of workers in the colonies. As we did not census the tested colonies, we cannot test for an effect of colony size on the number of patrolling ants in focal trees. We thus used the difference between the number of

![Fig. 1. Distribution map of the obligatory ant-plant symbiosis between *P. phylax* and *L. africana*, showing the two study sites (BON: N’kolobondé, EBO: Ebodjé). Hatching indicates area where the system is probably nearly extinct now, due to human activities (especially clearing of forests for plantation agriculture).](image_url)
workers induced by the damage on the experimental leaflet and the number of workers on the control leaflet to calculate ant response to damage in proportional terms and estimate colony behavioural investment in plant defence. For each minute of each test we computed the theoretical proportion of workers induced by the damage \( I \) as follows: \( I = (E - C)/(E + C) \), where \( E \) and \( C \) are the numbers of ants on the experimental and control leaflets respectively. This computation implicitly assumes that the number of ants on the control leaflet represents the constitutive level of ant patrolling activity which should also be observed on the experimental leaflet, any additional ants representing induced activity. We computed the mean of \( I \) for each test, and compared these values between the two study populations. We used non-parametric tests for comparing either independent or paired samples. All results were statistically analyzed using R 2.9.2 (R core development team).

3. Results and discussion

Our results showed that the number of ants was higher on the experimental than on the control leaflets (Fig. 2A; Wilcoxon signed-rank tests: BON, \( V = 9, P < 0.001 \); EBO, \( V = 22, P < 0.01 \), demonstrating that the experimentally damaged leaflets were attractive to workers of both populations. This indicates that in both populations behavioural traits of ants should provide significant protection to host plants. The number of patrolling ants did not differ significantly between populations for both the experimental and control leaflets (Fig. 2A; Mann–Whitney–Wilcoxon tests;
experimental: $W = 164, P = 0.33$; control: $W = 168.5, P = 0.39$). However, the difference in the number of ants between experimental and control leaflets was higher in N’kobondé than in Ebođé (Fig. 2A; Mann–Whitney–Wilcoxon test: $W = 124, P < 0.04$). Similarly, when considering the proportion of workers induced by the damage ($t$), investment in defence was significantly less pronounced in the population of Ebođé, at the southern edge of the range (Fig. 2B; Mann–Whitney–Wilcoxon test: $W = 310, P = 0.01$). Moreover, the lag time before workers returned to manipulated leaflets was significantly higher in Ebođé (Fig. 2C; Mann–Whitney–Wilcoxon test: $W = 120, P = 0.03$). Thus, the two populations differed in the behavioural investment of individual workers in host-plant defence. Contrary to our initial expectation, ant colonies at the colonization front did not compensate for lower investment in number of workers by more effective protective behaviour of each worker. Instead, individual workers responded less effectively to leaf damage at the colonization front. Seasonal climatic variations cannot account for the difference observed between the two sites because all the tests were performed within 10 days during the dry season, in March 2009. Rain strongly inhibits ant activity, but this could not account for the site effect because it did not rain during the test days.

Range expansion of the symbiosis appears thus to have affected not only the life history traits of *P. phylax*, but also ant behavioural traits. The lower allocation to worker production at the colonization front is accompanied by lower investment in protective behaviour by individual workers. Range expansion seems to have selected for an ant strategy that is less mutualistic as a whole. Reduced investment in defence could result from lower herbivore pressure, but preliminary experiments indicated that herbivore pressure in the studied populations is highly variable in time and space, even at a very small scale (Debout, 2003), suggesting it does not account for our results. The proximal cause of the lower per-worker investment in protective behaviour observed at the colonization front remains to be elucidated, but we hypothesize that it could be a consequence of the greater investment in reproduction. As alates of *P. phylax* are much larger than workers, sexual brood requires more cleaning and food provisioning than worker brood, and we expect colonies producing proportionally more sexual (and larger sexuals) to devote more worker behavioural investment to brood care. According to the fixed threshold model for regulation of division of labour (Bonabeau et al., 1996; Theraulaz et al., 1998), worker behavioural castes are determined by variation in response thresholds between individuals: for instance, those workers which have a lower response threshold to brood stimuli become nurses. As the intensity of a stimulus increases, however, it reaches the response threshold of workers that would otherwise not respond to it. As a consequence, a more demanding brood results in an increase of the nurse caste. A higher investment in sexuals, as observed in the colonies of *P. phylax* at the leading edge of its range, could thus result in proportionally fewer workers engaged in protection of the plant.

Other ant species obligatorily associated with *L. africana* would be expected to respond to range expansion the same way as *P. phylax*. Thus, a prediction from our results is that the parasitic ant, *C. mckeyi*, would have a more parasitic behaviour on the colonization front than in the median part of the distribution. This could take the form of some kind of host plant protection in the latter region. We were not able to investigate in detail the behaviour of this ant in the focal sites but field observations revealed that some colonies patrol the young leaves of their host plant in the median part of the distribution, a behaviour not recorded at the southernmost edge of the range. Several life history traits further suggest that the less dispersive colonies of *C. mckeyi* at BON have shifted from strong to weaker parasitism. At BON, colonies of *C. mckeyi* delay reproduction to a larger colony size and have larger and longer-lived colonies than at EBO (Debout, 2003; Debout et al., 2009; Léotard et al., 2009).

The conflict over reproduction in horizontally transmitted symbioses is well illustrated by the occurrence of castration parasites of ant–plants (Yu and Pierce, 1998). Although *Alomerus* ants protect * Cordia nodosa* plants, they are parasitic because they cut floral buds, causing the plant to invest more in vegetative parts, and thus in nesting space and food for the ant (Frederickson, 2009). The mutualistic features of the plant are maintained because protective, non-castrating ant species compete with the castrating partner (Yu et al., 2001). In contrast, in *L. africana, P. phylax* is the sole protective ant. Strongly parasitic tendencies at the colonization front may thus be counter-selected. Indeed, inability of a host to “punish” could lead to over exploitation (cheating) by the symbiont, as recently shown in the ant–plant symbiosis involving the plant *Korthalsia furttadouma* (Edwards et al., 2010). “Sanctions” exerted on ants by the plant are thus expected either to evolve in response to cheating, or to appear as simple ecological consequences of increased herbivory. The number of domatia and extrafloral nectaries produced is likely to be reduced by herbivory. Such “sanctions” have been evidenced in the plant *C. nodosa*: production of domatia decreased after an experimental increase of herbivory (Edwards et al., 2006). However, this reaction can also be interpreted in the context of partner fidelity feedback (Weyl et al., 2010). Responses of *L. africana* to less mutualistic *P. phylax* at the colonization front, either by local adaptations to cheating ants or as a consequence of increased herbivory, could include reduced production of extrfloral nectar and greater relative investment in reproduction. This last feature could also be a consequence of selection for a more dispersive phenotype of the host plant at the colonization front. However, Léotard et al. (2009) detected no gradient in the number of nectaries or in tree size at first reproduction (a trait that should be related to dispersal capacity) of *L. africana* near the southern edge of its range. The absence of “sanctions” applied by the plant could be due either to lower herbivory rate at EBO, balancing the effect of lower protection on mutualistic features, or to the fact that the generation time of the plant is much longer than that of the ant, leading to slower local adaptation at the colonization front. Finally, ant phenotypes tending to parasitism are likely to be transitory in a given plant population. As the colonization front progresses, their replacement by highly mutualistic phenotypes—which are likely to be competitively superior due to larger worker numbers and to polygyny—may not leave enough time for the plant to evolve counter-adaptations. Edwards (2009) proposed that tolerance might maintain cooperation in mutualisms. The symbiosis between *L. africana* plants and *P. phylax* ants might be an example in which host tolerance to temporary cheating by the symbiont has a stabilizing effect on the mutualism. In this system, tolerance is beneficial because the long-lived host will have opportunities to interact with many generations of symbionts, most of which will be highly beneficial.

According to the theory of coevolution in geographical mosaics (Gomulkiewicz et al., 2000; Thompson, 1999), the outcome of coevolution shows geographic variation across populations. Limited gene flow allows for local adaptations, leading to a spatial mosaic of outcomes. The outcome is usually considered to be determined by ecological and environmental factors (Hernandez and Barradas, 2003; Thompson and Cunningham, 2002). The interaction between *Leonardoxa* and *Petalomyrmex* shows variable outcomes among populations. The association is highly mutualistic in the centre of its distributional range, but the ant shows a strategy closer to the ‘parasitic’ part of the mutualism–parasitism continuum (Ewald, 1987) at the colonization front. Similarly, *C. mckeyi* appears
to be a more "virulent" parasite at the colonization front. Thus, range expansion also can be a factor in mosaic coevolution.

We provide, to our knowledge, the first evidence for geographical variation in a mutualistic behavioural trait driven by range expansion. Integrating population history and geographic mosaics of coevolved traits could lead to major advances in the evolutionary ecology of interacting species (Alvarez et al., 2010). Our finding of variation in intensity of investment in a mutualistic behavioural trait, combined with the reconstruction of population history (Dalecky et al., 2007; Léotard et al., 2009), shows that the stability of mutualism can potentially be affected by distribution shifts. Although our results require replication in other sites before we can reach broader conclusions, they open new perspectives into fundamental aspects of the dynamics of interspecies interactions. Studies of ant–plant symbioses could help us understand the consequences of global climate change for the structure and functioning not only of mutualisms, but of the ecological communities in which they are ensnared.

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