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Ilkka Hanski: The legacy of a multifaceted ecologist

Evolution of adaptive phenotypic plasticity in male orange-tip butterflies

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The evolution of adaptive phenotypic plasticity, whereby organisms respond to cues to produce phenotypes appropriate to their future environment, is not well understood. Male orange-tip *Anthocharis cardamines* butterflies utilizing the host-plant lady's smock *Cardamine pratensis* (on which food is limiting) are smaller and emerge earlier than those utilizing garlic mustard *Alliaria petiolata*. Since small size reduces dispersal rate, this coupling produces an adaptive 'emerge early and wait' mate location strategy. Here we show that early emergence is abolished when males are subjected to a severely restricted diet, which we take to be the ancestral response. We propose that the derived (early emergence) response on *C. pratensis* has evolved through sex-specific changes in the timing and efficiency of developmental and metabolic processes in late larval life. Our results offer broad support to the idea that adaptive phenotypic plasticity can evolve through the selection of cue-sensitive modifier genes controlling initially passive plastic responses.

Introduction

Ilkka Hanski was deeply interested in eco-evolutionary feedbacks, particularly within the context of metapopulation dynamics (Hanski 2011). In the Glanville fritillary (Åland system), together with Mike Singer and Mikko Kuussaari, he demonstrated that a cline for oviposition preference (Kuussaari *et al*. 2000), interacting with local host-plant composition (*Plantago lanceolata* and *Veronica spicata*), had a significant impact on gene flow and founder events (Hanski & Singer 2001, Hanski 2011). As a tribute and complement to this work, we here examine how the divergent quality of alternative hosts with contrasting distributions across the British landscape may have led to the evolution of adaptive phenotypic plasticity in the orange-tip butterfly.

Phenotypic plasticity refers to the production of different phenotypes in different environments by the same genotype. The way in which the realized phenotype varies along an environmental gradient is called the reaction norm of the genotype (Pigliucci *et al*. 2006, Ghalambor *et al*. 2007, Fusco & Minelli 2010). Reaction norms may differ among genotypes, enabling selection to modify plastic responses within populations over time and among populations across space (de Jong 2005, Crispo 2007, Ghalambor *et al*. 2007). Plastic responses may be critical in allowing organisms to invade new environments by shifting their expressed phenotypes

closer to the peak of the new adaptive landscape (Price *et al*. 2003, Crispo 2007, Ghalambor *et al*. 2007). In a new habitat, elements of the ancestral environment will still be present (e.g. predators, prey, or abiotic conditions). The frequency with which these are encountered will determine how the plastic responses evolve (Pigliucci *et al*. 2006). In the presence of ancestral cues, selection will be constrained to modifying the slope and intercept of the reaction norms (de Jong 2005, Crispo 2007), whereas in their absence, it may lead to genetic assimilation of an adaptive response or suppression of a maladaptive one. Genetic assimilation involves the canalization of the plastic response into a genetically determined one, in which the new phenotype is produced in the absence of the previously required environmental stimulus (Pigliucci & Murren 2003, West-Eberhard 2003, Pigliucci *et al*. 2006); it may be particularly favoured if the costs of maintaining plasticity are high (Price *et al*. 2003, Pigliucci *et al*. 2006, Crispo 2007, Fusco & Minelli 2010). Genetic compensation is the reverse of genetic assimilation, in which a plastic response is suppressed in a new environment (Grether 2005).

The frequency with which elements of a former environment are encountered will depend on how permanent a change is in time and on how uniform it is across space. In heterogeneous landscapes, uniformity will be effectively determined by how quickly the habitat changes with respect to the dispersal ability of the organism. Phenotypic plasticity has the potential to track changes across landscapes and through time, allowing organisms to respond quickly to their immediate environment. For this reason, adaptive phenotypic plasticity is predicted to evolve in environments with high spatio-temporal heterogeneity (Leimar 2009, Scheiner 2013). A high dispersal rate between demes also favours plastic responses over genetic differentiation, at least when selection acts before dispersal, i.e. when selection takes place in the same environment in which the organism is phenotypically determined (Leimar 2009, Schiener & Holt 2012, Scheiner *et al*. 2012).

The evolution of adaptive phenotypic plasticity, in which the reaction norms of an organism are accurately adjusted to maximize its fitness in alternative environments, depends on the existence of reliable cues (Iwasa & Haccou 1994, Leimar 2009, Fusco & Minelli 2010, Forsman 2015). If an environmental stimulus is strongly correlated with the shape of the future adaptive landscape to which the organism will be exposed, then various plastic responses may be co-opted to produce a highly integrated response to that stimulus. Hence, a key signature of adaptive phenotypic plasticity is that the response is anticipatory, and follows indirectly from the coordinated up- or down-regulation of genes modifying a suite of developmental processes in response to an environmental cue (Nijhout 2003, Fusco & Minelli 2010, Forsman 2015). The mechanism(s) by which this could evolve from a set of initially uncoordinated direct physiological responses to a novel environment are therefore likely to involve the selection of cue-sensitive modifier genes controlling the developmental system (Leimar 2009). In insects this would most likely involve modification of the endocrine system (Nijhout 2003).

In phytophagous insects, utilization of a new host-plant corresponds to the invasion of a novel environment (Grether 2005). If new and old host-plants occur sympatrically, then the landscape will be highly heterogeneous from the point of view of host-type availability. Hence, any plastic responses associated with the new host-plant cannot be subject to genetic assimilation or compensation, unless the old host-plant is abandoned. In these circumstances, selection will be limited to altering the reaction norms underlying the plastic responses. Since the new host is likely to contain an array of chemical compounds that the insect has not previously encountered, there are enhanced opportunities for one (or more) of these to act as a cue for the developing larva. If the host-plant is associated with a specific ecological niche, then the larva will receive information not just about its future diet, but also about the selective regime it will encounter as an adult.

The pierid butterfly *Anthocharis cardamines* (the orange-tip) is highly polyphagous on brassicaceous hosts in continental Europe, although in Britain it is more strongly associated with the two host-plants *Alliaria petiolata* (garlic mustard) and *Cardamine pratensis* (lady's smock). The

butterfly is protandrous (males emerge before females) and exhibits female-biased sexual size dimorphism (SSD) (females are larger than males). Males which have utilized *C. pratensis* as larvae tend to be smaller, emerge earlier, and disperse more slowly than those which have utilized *A. petiolata*, at least in a study population in Cheshire, northwest England (Davies & Saccheri 2013). Davies and Saccheri (2013) proposed that these phenotypically plastic responses are adaptive, and together contribute to an "emerge early and wait" mate location strategy. This is possible because *C. pratensis* tends to be associated with compact high density *A. cardamines* populations from which dispersal is disadvantageous and in which early emergence is favoured in the intense scramble competition for mates. Hence, *C. pratensis* is envisioned as acting as a cue for the type of environment into which males will emerge.

A potential problem in the study of the evolution of phenotypic plasticity is the difficulty of catching it in the act, since it is predicted to evolve quickly (Pigliucci & Murren 2003, Grether 2005). Hence, most studies rely on inferences based on differences between ancestral and derived populations. We here take a novel approach to this problem. We hypothesized that, ancestrally, utilization of *C. pratensis* led to the direct depression of body size through undernourishment. This passive (uncued) response would have been stressful and unlikely to have been accompanied by early emergence, which is clearly anticipatory. We therefore compared the emergence timing of specimens reared on unrestricted and restricted diets, to see if the hypothesized ancestral response could be recovered in the latter. For this to work, the restricted diet treatment would have to be harsher than the average diet to which larvae are regularly exposed on *C. pratensis*, and to which (we hypothesize) they are now adapted. We also examined how larvae respond to the size variation of *C. pratensis* plants in the wild, and whether this impacts the emergence timing of the resultant imagines, to see how far the plastic responses can accommodate the nutritional challenges commonly encountered on this host, and to assess whether further evolution of them is possible.

Methods

The developmental response of *A. cardamines* to a restricted diet was analysed for individuals within the same family ($N = 20$), to minimize genetic variance in the traits of interest (wing-length and eclosion day). All larvae were reared through the 4th instar on *A. petiolata*, then assigned at random to restricted or unrestricted diets for the fifth (final) instar. In the restricted diet treatment, larvae were divided between *A. petiolata* and *C. pratensis*, and food was withdrawn when they reached 25 mm in length. This is below the usual minimum length (26 mm) of full-fed larvae in the wild (Davies & Saccheri 2013: fig. 3), and was therefore expected to trigger a stressed response. In the unrestricted diet treatment, larvae were reared *ad libitum* on *A. petiolata* until pupation. The effect of host plant on wing length and emergence timing in full-fed specimens was investigated within families by rearing larvae *ad libitum* on *A. petiolata* or *C. pratensis* until pupation.

All studies on wild material were undertaken in Dibbinsdale Nature Reserve, Cheshire, northwest England. The Reserve was divided into seven sub-sites (for a map *see* Davies & Saccheri 2013) in which one or both host plants were abundant and where *A. cardamines* males, which utilize the host plants as nectar sources, were easily collected.

To investigate inter-deme variation in *C. pratensis* plant-height, specific transects were selected at each sub-site along which all plants were measured. Each ramet was measured to the nearest 1 cm at the time of flowering, and marked with permanent marker pen to prevent resampling. Each transect was visited every few days until an adequate number of ramets (~50) had been sampled. Since mean plant-height varied continuously between sub-sites, the distinction between the "small" and "large" ecotypes of *C. pratensis* (introduced by Davies & Saccheri 2013) was weakened, although the "large" ecotype did produce the tallest plants. For the purposes of the current analysis, the ecotype distinction is therefore dropped.

Wild *A. cardamines* larval body-lengths were obtained from the largest value in the sequence of daily measurements through the fifth instar of

the distance between the tip of the head capsule and the tip of the anal flap. Wild adult wing-lengths were obtained by immobilizing specimens between the folds of a butterfly net and measuring the distance between the base and the apex of the underside of the left forewing; after individual marking and release, all dates of subsequent recapture were recorded.

All statistical analyses were undertaken in SPSS ver. 22. For multivariate general linear models, Levene's test for homogeneity of variances and Box's test for homogeneity of covariances were undertaken before the analyses.

Results

The restricted diet treatment caused high mortality (43% of larvae subjected to it failed to pupate). Among the survivors, host plant had no effect on either emergence timing or wing-length; this factor was therefore omitted from the analysis. A multivariate general linear model showed that there were significant interactions between sex and diet on both emergence day and wing length (Table 1). The restricted diet treatment retarded the emergence of males but not females, to the extent that protandry was abolished (Fig. 1a). It also depressed wing-length in both sexes, but more so in females than males, to the extent that female-biased SSD was also abolished (Fig. 1b).

The loss of female-biased SSD was an effect of starvation rather than reduction in size *per se*, since in the absence of starvation laboratory reared females were larger than males on both *A. petiolata* and *C. pratensis* in spite of the size differences produced by these hosts (Table 2). Underfed specimens (both sexes) suffered reduced longevity as adults compared with full-fed ones (Table 3). This is likely to be a size rather than starvation mediated effect, since in the field the survival curves of small specimens, the majority of which are unlikely to have been starved (*see* below), show a characteristic senescence phase, whereas those of larger specimens do not (Fig. 2).

The effect of *C. pratensis* on emergence day and wing length in full-fed specimens within families was analysed in a multivariate general linear model for each sex, with family and host-plant as independent variables (Table 4). Specimens of both sexes are significantly smaller and males emerge significantly earlier when reared on *C. pratensis* than on *A. petiolata* (enhanced protandry). There is currently no evidence that host plant affects female emergence timing, although this conclusion must be treated with

Table 1. A multivariate general linear model for the effects of sex and diet on emergence day and wing length in *Anthocharis cardamines*.

Source	Dependent variable	Type III SS	df	MS	*F*	*p*
Corrected model	Emergence day	26.979[a]	3	8.993	7.525	0.006
	Wing length	117.640[b]	3	39.213	36.591	< 0.001
Intercept	Emergence day	1268.667	1	1268.667	1061.646	< 0.001
	Wing length	4177.153	1	4177.153	3897.810	< 0.001
Sex	Emergence day	10.381	1	10.381	8.687	0.015
	Wing length	.972	1	.972	.907	0.363
Diet	Emergence day	6.329	1	6.329	5.296	0.044
	Wing length	117.569	1	117.569	109.707	< 0.001
Sex × diet	Emergence day	6.329	1	6.329	5.296	0.044
	Wing length	8.920	1	8.920	8.323	0.016
Error	Emergence day	11.950	10	1.195		
	Wing length	10.717	10	1.072		
Total	Emergence day	1419.000	14			
	Wing length	4557.000	14			
Corrected total	Emergence day	38.929	13			
	Wing length	128.357	13			

[a] R^2 = 0.693 (adjusted R^2 = 0.601), [b] R^2 = 0.917 (adjusted R^2 = 0.891).

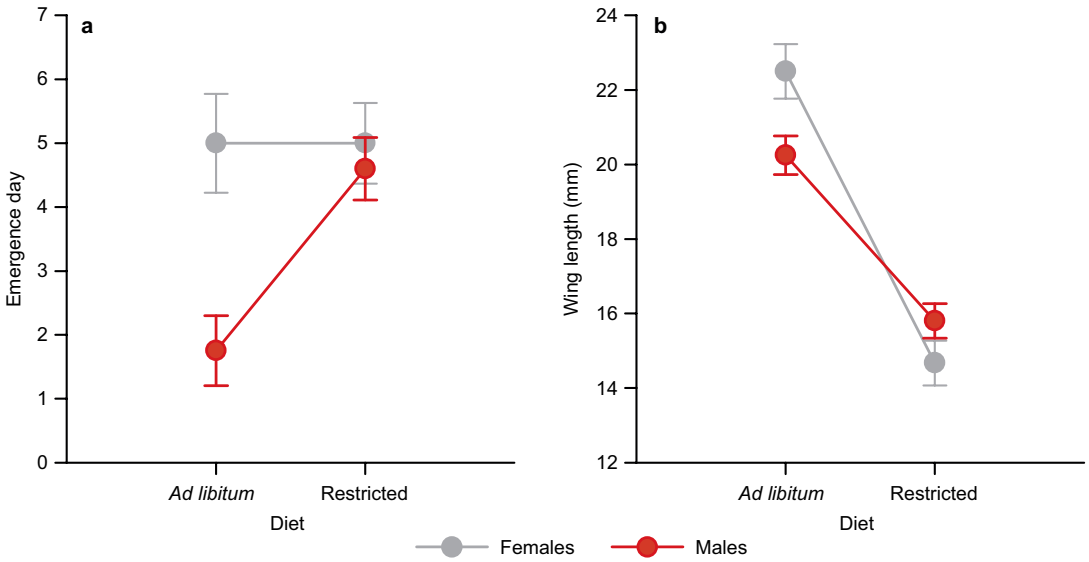

Fig. 1. The effect of the interaction between diet treatment and sex on (**a**) emergence timing and (**b**) wing length in *Anthocharis cardamines* (marginal means ± SE).

caution as only 7 females from 4 families were reared from *C. pratensis*.

The average size (body length) of wild *A. cardamines* larvae increased with the average height of *C. pratensis* plants among sub-sites in Dibbinsdale (Fig. 3). Hence, nutrition (indexed by plant height) is a limiting factor on this host; if it were not, larvae would always attain full size. This confirms that in the absence of an adaptive response larvae would be highly physiologically stressed through nutritional deprivation on *C. pratensis*. To test whether the Dibbinsdale population of *A. cardamines* has evolved to cope with the extreme nutritional challenge presented by these plants, the emergence timing of males at the sub-site with the smallest average-sized plants and larvae was examined over a four year period. Regression analysis demonstrated

Table 2. Maintenance of female-biased sexual size dimorphism among host plants in full-fed (*ad libitum*) *A. cardamines* (for the difference between males and females on *A. petiolata*, $t_{40} = 2.99$, $p = 0.005$; for the difference on *C. pratensis*, $t_{26} = 2.79$, $p = 0.01$).

Host plant	Sex	*N*	Mean wing length (mm) ± 95%CI
A. petiolata	Males	22	20.21 ± 0.47
	Females	20	21.15 ± 0.46
C. pratensis	Males	19	17.80 ± 0.55
	Females	9	19.12 ± 0.96

Table 3. Survival of *A. cardamines* imagines reared on a normal (*ad libitum*) or restricted diet (host plant withdrawn when larvae reached 25 mm).

Treatment	Mean emergence day	*N*	Mean wing length (mm)	Percentage still alive on 16 May	Percentage still alive on 19 May
Normal (*ad libitum*)	26 April	31	20.2	97	90
Restricted diet	28 April	8	15.4	0	0

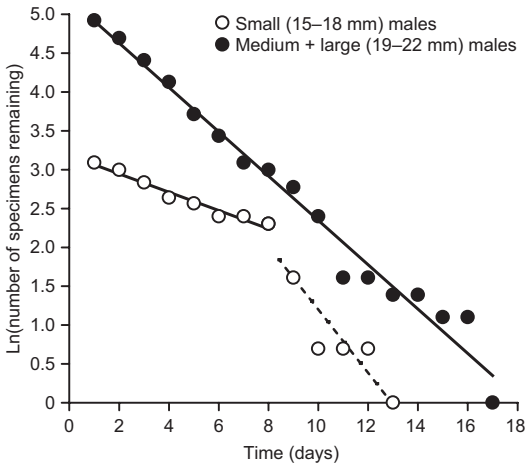

Fig. 2. Survival curves of small and medium/large sized (wing length) male *A. cardamines* in Dibbinsdale from combined mark–release–recapture data for 2005–2007. Small specimens exhibit a distinct senescence phase after 8 days (dashed line) whereas larger specimens do not. The pre-senescence daily survival rate of small specimens is higher, which Davies and Saccheri (2013) attributed to a slower dispersal rate (note that the term "survival" as used here includes the effects of dispersal as well as death).

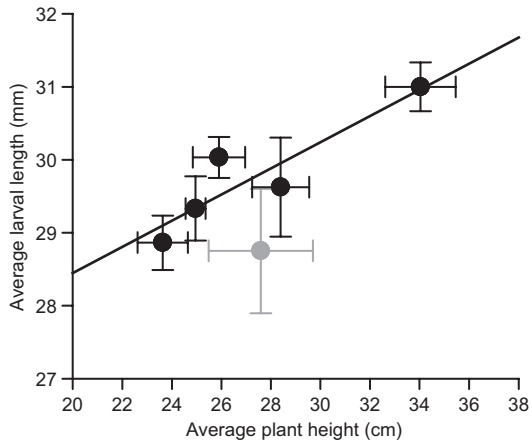

Fig. 3. Average length of full-grown larvae (± SE) as a function of average *C. pratensis* plant height (average of yearly means ± SE) in five sub-sites at Dibbinsdale in 2008–2011 (a sixth sub-site (shown in grey) has been excluded from the regression since only 4 mature larvae were sampled there). Regression equation: Larval length = 0.18 × (plant height) + 24.86, R^2 = 0.83, p = 0.03.

that small butterflies emerged significantly earlier than large ones (Table 5). When the flight season was split into early and late time periods, the majority (75%) of males with wing lengths ≤ 18 mm, which are most likely to have resulted from the small larvae produced on *C. pratensis*, emerged during the early time period (Fig. 4); the difference in the proportion of specimens emerging in early and late season with wing lengths ≤ 18 mm and ≥ 19 mm is highly significant (χ^2 = 13.70, df = 1, p = 0.0002). This suggests that small specimens were not stressed, and that the butterfly has indeed evolved to cope with the nutritional challenge presented by the smallest average-sized host-plants in the Reserve.

Table 4. Contrast results (*k* matrix) for the effect of *C. pratensis* compared with *A. petiolata* on emergence day and wing length in male and female *A. cardamines*. The results are taken from a multivariate general linear model for each sex on the main effects of family and host-plant on emergence day and wing-length, so the effect of family has been controlled for. For males, there were 6 families and a total of 19 specimens were reared on *C. pratensis* and 22 on *A. petiolata*; for females there were 4 families with 7 specimens reared on *C. pratensis* and 18 on *A. petiolata*.

Host difference contrast	Males		Females	
	Emergence day	Wing length	Emergence day	Wing length
Contrast estimate	–1.160	–2.223	–0.194	–1.920
Hypothesized value	0	0	0	0
Difference (estimate – hypothesized)	–1.160	–2.223	–0.194	–1.920
SE	0.395	0.398	0.554	0.505
p	0.006	< 0.001	0.730	0.001
95%CI for difference				
lower bound	–1.963	–3.031	–1.349	–2.973
upper bound	–0.358	–1.414	0.962	–0.867

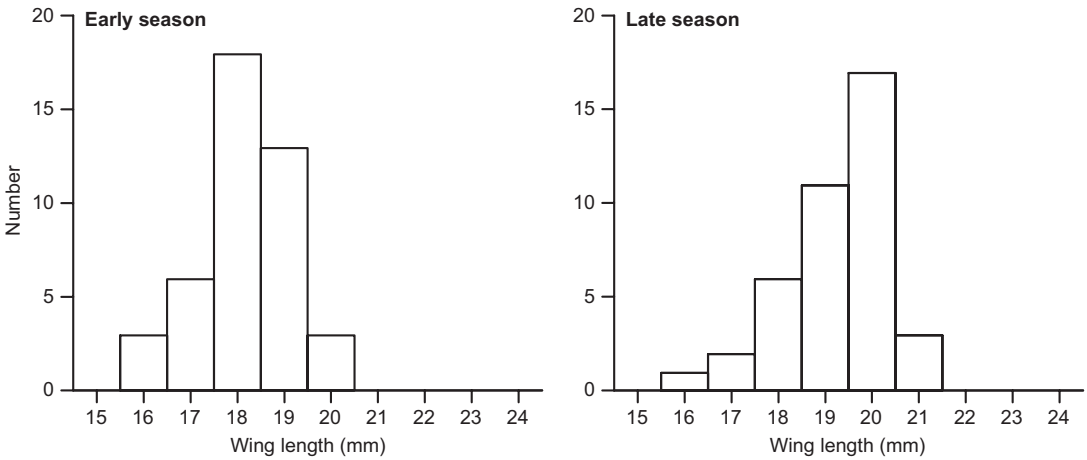

Fig. 4. Male early and late season wing-length distributions in 2009–2012 at the sub-site in Dibbinsdale where *C. pratensis* plants and larvae were smallest. In each year, the whole season sample was divided into early and late season sub-samples, which were then separately combined over the years. Early season mean wing-length (± 95%CI) = 18.16 ± 0.31 mm, average date of first capture of each specimen = 20 April; late season mean wing length = 19.25 ± 0.36 mm, average date of first capture = 3 May.

Discussion

Ancestral *A. cardamines* utilizing small *C. pratensis* plants were probably highly physiologically stressed by undernourishment, with consequent depression of body size and abolition of protandry and sexual size dimorphism (Fig. 1). The derived population of *A. cardamines* in the study area still exhibits reduced body-size on *C. pratensis*, but SSD has been restored and protandry has been enhanced (Tables 2 and 4), consistent with modification of the initial reaction norms and evolution of the plastic response. Since ancestral host-plants are still regularly encountered, the response has not been genetically canalized; that is, the ancestral response is still exhibited on larger host-plant species for which food is not limiting. Instead, modification of the response on *C. pratensis* has likely involved the selection of modifier genes controlling the developmental system (Leimar 2009). Since the altered regulation of these genes occurs in response to a cue associated with *C. pratensis*, and since early male emergence is favoured on this host plant (Davies & Saccheri 2013), an initially passive and maladaptive plastic response (small size, late emergence) has become anticipatory and adaptive (small size,

Table 5. Regression analysis on the effects of wing length and year (2009–2012) on the emergence date of male *A. cardamines* at the sub-site in Dibbinsdale where *C. pratensis* plants and larvae were smallest (after backward deletion of non-significant years). For consistency, the study period is displaced one year later than the period for which larval size was assessed (Fig. 3), since the imagines emerge the year after larval feeding. Dependent variable: Emergence date.

	Unstandardized coefficients		Standardized coefficients	*t*	*p*	Correlations			Collinearity statistics	
	B	SE	β			Zero	Partial	Part	Tolerance	VIF
Constant	42439.5	15.886		2671.5	< 0.001					
Wing length	2.321	0.843	0.261	2.754	0.007	0.197	0.296	0.259	0.983	1.017
2010	5.939	2.511	0.228	2.365	0.020	0.103	0.257	0.222	0.949	1.054
2012	15.372	2.888	0.515	5.323	< 0.001	0.442	0.514	0.500	0.945	1.059

early emergence). We now outline a working hypothesis to explain how this has happened.

The stressed response to undernourishment affects both emergence timing and body size in males, but only the latter in females, suggesting that early male emergence is metabolically costly and dependent upon a nutritional input from the host plant. This is consistent with the utilization of stored energy reserves for post-diapause ontogenetic development in *A. cardamines* pupae (Stålhandske *et al*. 2015), which is faster in males than females (Stålhandske *et al*. 2014); it is also in line with experiments demonstrating that larvae of multivoltine butterflies approaching pupation can more easily switch from diapause to direct development than in the reverse direction, which reveal the need for adequate physiological preparation in late larval life for post-metamorphic processes (Friberg *et al*. 2011). Since males are smaller than females (female biased SSD) in unstressed conditions, we propose that the extra energy required for early emergence is acquired at the cost of a larger body-size; that is, males divide their nutritional input between advanced emergence and growth, whereas females devote it entirely to the latter. The two sexes therefore diverge in nutritional allocation at some point during the final larval instar, before which both sexes use nutrition entirely for growth. In the ancestral condition, the divergence point occurs relatively late in the final instar (Fig. 5a). Hence, larvae are highly vulnerable to the ontogenetic effects of starvation, which arrests development before the two sexes are fully differentiated in either body size or (future) emergence timing (solid red line in Fig. 5a).

In the derived condition, the divergence point moves to an earlier point in final instar development in response to a cue from *C. pratensis* (Fig. 5b). This allows the two sexes to become fully differentiated with respect to both body size and emergence timing in spite of (what formerly amounted to) a lack of food, which now exceeds their nutritional requirements (solid red line in Fig. 5b). In addition, male emergence timing has been modified by the more efficient conversion of nutritional input into advanced eclosion. We therefore propose that the evolution of adaptive phenotypic plasticity in *A. cardamines* has required two changes: an advance in the point of ontogenetic divergence between the two sexes, and a change in the reaction norm controlling male emergence timing.

The results of the food limitation experiments can be explained on the assumption that nutrition was withdrawn before the ontogenetic divergence point had been reached on either host plant (dashed red lines in Fig. 5a and b). This resulted in an emergency response in which larvae pupated before they were sexually differentiated in terms of body size and emergence timing (though not in terms of colour pattern, which was close to normal for both sexes). This response could not always be achieved (the mortality rate was 43%) but the fact that it can be effected at all suggests that the species has repeatedly been exposed to starvation in the past and has evolved some resistance to it (Wiklund & Ahrberg 1978).

Intrinsic resistance to starvation probably aided initial establishment of *A. cardamines* on small *C. pratensis* plants. However, in order to successfully colonize this host, there must have been some actual fitness benefit(s) which prevented the evolution of oviposition avoidance. Although *A. cardamines* is highly polyphagous, there is evidence that oviposition avoidance can evolve in this species. Posledovich *et al*. (2015) report that whereas females readily oviposit on pre-reproductive and early bud stages of *Arabis glabra*, they avoid doing so on similar stages of *A. hirsuta*, since this negatively impacts larval performance. Repeated exposure to small *C. pratensis* plants would likely have had a similar effect if starvation risk had not traded-off with some compensatory advantage(s); this is supported by the fact that the smallest *C. pratensis* plants are avoided by females in contemporary populations (Dempster 1997, Arvanitis *et al*. 2008). Davies and Saccheri (2013) suggested that depressed dispersal of small undernourished males would have been advantageous in compact high density populations associated with *C. pratensis*. This downstream effect of body-size plasticity would have been crucial in enabling *A. cardamines* to invade a new host-plant environment by shifting its expressed phenotype closer to the peak of the new adaptive landscape associated with it (Price *et al*. 2003, Crispo 2007, Ghalambor *et al*. 2007).

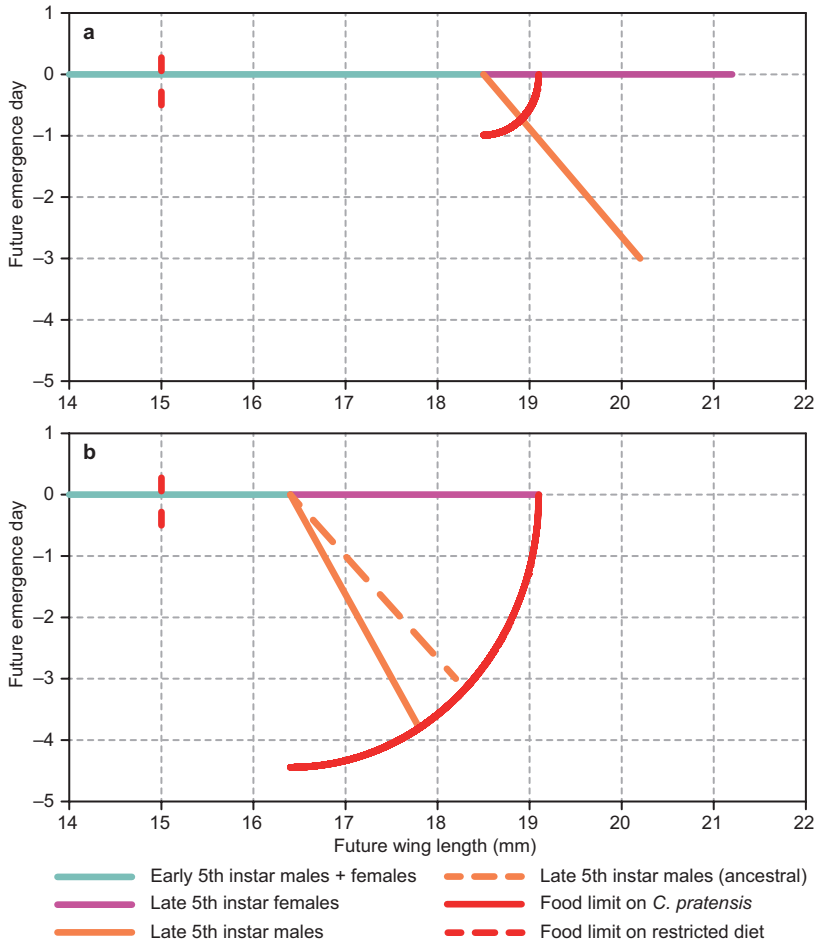

Fig. 5. Hypothesized pattern of ontogenetic development of male and female *A. cardamines* larvae in (**a**) ancestral and (**b**) derived populations. Lines represent how larvae allocate nutritional input to future (adult) body-size (wing-length) and emergence timing as the fifth (final) instar progresses (from left to right). Initially, both sexes allocate all resources to increasing body-size (blue line). At a certain point, males and females diverge in their allocation patterns; females (purple line) continue to allocate all resources to boosting body-size, whereas males (orange line) divide their nutritional input between increased body size and early emergence. This results in female-biased sexual size dimorphism (larger females) and protandry (earlier emergence of males). In the ancestral condition, the ontogenetic divergence point occurs relatively late in the fifth instar on all host-plants; hence, when individuals run out of food on *C. pratensis* (red line), arrested development prevents full sexual differentiation (in terms of body-size and emergence timing). In the derived condition, exposure to *C. pratensis* shifts the divergence point to an earlier stage of ontogeny, enabling the two sexes to become fully differentiated before they run out of food (red line). In addition, the efficiency with which males utilize nutritional input to advance their emergence timing has been increased (solid orange line; the dashed orange line shows the equivalent ancestral condition transposed to the new environment). Sexual size dimorphism and protandry can be abolished in both environments by withdrawing nutrition very early in the fifth instar (dashed red lines), as happened in our experiments. The diagrams are parameterized to accurately reflect our laboratory results in terms of differences of size (Table 2) and emergence timing (Davies & Saccheri 2013: fig. 5 & table 3) on *A. petiolata* and *C. pratensis*.

Hence, selection against oviposition on *C. pratensis* would have been reduced, allowing time for selection to modify the reaction norms underlying the plastic response.

The advance in timing of the ontogenetic divergence point between the sexes in how they allocate nutritional input to body size and future emergence timing has probably involved modi-

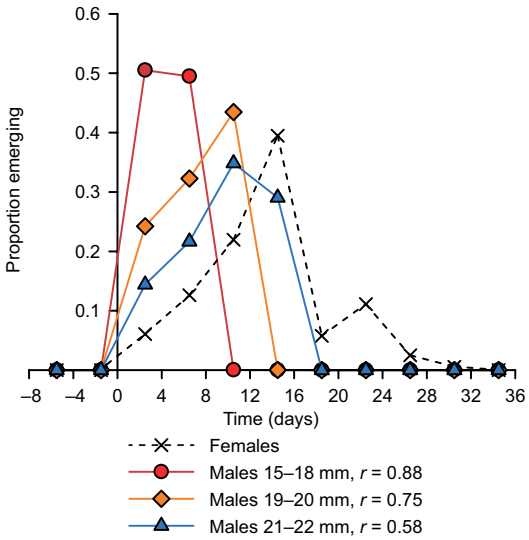

Fig. 6. Predicted emergence curves for different sized (wing length) *A. cardamines* males, calculated from the observed female emergence curve in Dibbinsdale Nature Reserve in 2007 (from Davies & Saccheri 2015) using the Parker and Courtney (1983) simulation model, on the assumption that their residence rates *r* (= proportion of specimens remaining in the Reserve per day), which are determined by both survival in and dispersal from the Reserve, can be equated wholly to the survival rate in the model.

fication of the pattern of hormonal regulation in final instar larvae. In insects, the hormones controlling distinct plastic forms (polyphenisms) are ecdysone and juvenile hormone, which also control moulting and metamorphosis (Nijhout 2003). All known cases of polyphenic switching between alternative developmental pathways occur in response to an environmental cue controlling the timing of hormone secretion, the timing of a hormone sensitive period, or the threshold of hormone sensitivity (Nijhout 1999, 2003). It therefore seems likely that the switch in timing of the ontogenetic divergence point on *C. pratensis* is a hormonally controlled response to a cue associated with that host plant. This could have evolved if dietary factors unique to *C. pratensis* happened to modify hormonal regulation in ancestral *A. cardamines* larvae; genetic variance in the magnitude of this effect (due to modifier genes) would then have allowed selection to move the divergence point to a safe distance from the starvation threshold. The same dietary factors would then act as a cue for an anticipatory phenotypic switch, the evolution of which would have restored protandry and SSD automatically, since the two sexes would then have adequate time to differentiate properly on *C. pratensis* (dashed orange line in Fig. 5b). The enhanced protandry elicited by exposure to *C. pratensis* in modern populations (solid orange line in Fig. 5b) could then have evolved through selection of further modifier genes controlling the efficiency with which nutritional input is converted into an advancement in eclosion timing in males. The process envisioned here therefore supports Leimar's (2009) view that adaptive phenotypic plasticity evolves through the selection of genes modifying initially passive plastic responses.

The evolution of protandry in insect populations is dependent on female monogamy (monandry), so that it is costly for males to emerge after their competitors in the scramble competition for mates. Game theoretic models on the evolution of protandry predict that it should increase in response to a high male survival rate and high population density (Wiklund & Fagerström, 1977, Bulmer 1983, Iwasa *et al.* 1983, Parker & Courtney 1983, Zonneveld & Metz 1991, Davies & Saccheri 2015). Due to its restriction to damp habitats, *C. pratensis* is discontinuously distributed across the British landscape, though it is often abundant where it occurs. From the point of view of *A. cardamines* males, larval exposure to this host plant therefore predicts that they will emerge in an isolated habitat patch with a high density of locally emerging females. Rapid emigration from such a patch would be clearly disadvantageous; in this case, dispersal can be taken to act analogously to death in game theoretic models (Davies & Saccheri 2013). The depressed dispersal of small males is therefore favoured; in order to fully exploit their advantage, models indicate that they should emerge earlier on average than their larger, faster dispersing competitors (Fig. 6). We believe this to be the selection pressure favouring earlier male emergence on *C. pratensis*. Due to its occurrence in both wet and dry habitats, and especially along roadside verges, *A. petiolata* is more continuously distributed across the British landscape; given its more frequent association with areas of low population density, a

later male emergence timing is favoured on this host plant.

Most game theoretic models on protandry assume that male emergence timing evolves in response to female emergence timing (Wiklund & Fagerström, 1977, Bulmer 1983, Iwasa *et al*. 1983, Parker & Courtney 1983, Zonneveld & Metz 1991), rather than the reverse (but *see* Fagerström & Wiklund 1982). Our experimental findings support this assumption for *A. cardamines*; since food limitation retards the emergence of males, protandry has likely evolved through a nutrient-dependent ramping-up of the same metabolic processes that control emergence timing in females. Our findings also offer an explanation for the association between female-biased SSD and protandry in diapausing pierid and satyrid butterflies uncovered by Wiklund and Forsberg (1991): males are smaller and emerge before females due to a trade-off in late larval life between the allocation of nutritional resources to body size and future emergence timing. This contrasts with the assumption that the association is due to a negative correlation between pupal size and morphogenetic development rate (Wiklund & Forsberg 1991), which has already been shown to be incorrect for *A. cardamines* (Davies & Saccheri 2013). However, Wiklund and Solbreck (1982) found that protandry is maintained by increased female pupal development time in the diapausing generation of the bivoltine pierid butterfly *Leptidea sinapis* and by decreased male larval development time in the non-diapausing generation. Hence, the developmental mechanism producing protandry varies across species and its control can switch between sexes even within the same species. It would be interesting to see how widespread the mechanism uncovered for *A. cardamines* is by applying the methods outlined here to other diapausing and non-diapausing species in all families.

A. cardamines is clearly well adapted to the dietary challenges presented by *C. pratensis* at our study site in northwest England. The sub-site with the smallest average-sized plants also produces the smallest average-sized larvae (Fig. 3), but small male butterflies resulting from them emerge early (Fig. 4), demonstrating that they are physiologically adapted to the reduced diet. However, the survival curves of small males generally exhibit a distinct senescence phase whereas those of larger specimens do not (Fig. 2). This does not mean that the latter specimens do not undergo senescence, but rather that it cannot be detected during their tenure in the Reserve; hence, they are physiologically stronger than the small specimens. The shorter life-span of dwarf males has not (yet) been ameliorated by selection, probably because the selection pressures favouring a longer life-span are weak. Since early emergence timing is so important for these specimens, it follows that an extension of life will have relatively little impact on their fitness. Moreover, there may be a trade-off between protandry and life-span, since early emergence is achieved by diverting nutritional input to it, which could otherwise have been used to boost longevity.

In summary, our results provide some support for currently held views regarding the evolution of adaptive phenotypic plasticity, including the role of passive plasticity in aiding initial establishment in a new environment (host plant) by reducing the fitness costs of exposure to it (preventing starvation) and gaining the organism time to adapt to it by shifting its phenotype closer to the new adaptive peak (mate-seeking advantage of slow dispersing males), the selection of modifier genes (controlling hormonal regulation and the efficiency of nutrient assimilation) associated with environmental cues (novel host-plant dietary factors) in restoring (circumventing nutrient limitation) and boosting (modulating emergence timing) fitness in that environment, and the importance of downstream effects of plasticity (depressed male dispersal) in a predictable environment (compact high density demes) in enabling an integrated anticipatory response to evolve in relation to upstream environmental cues (host-plant species).

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