Plastic responses of some life history traits and cellular components of body size in *Aphidius ervi* as related to the age of its host *Acyrthosiphon pisum*

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Phenotypic plasticity of wing size and shape has been evaluated in *Aphidius ervi* developing in its host, *Acyrthosiphon pisum*, parasitized at seven different ages. The parasitoid wing size was used as an estimator of both whole body size and its cellular composition. No size difference was observed in *A. ervi* adults emerged from aphids 1, 2 or 3 days old at parasitization. Body size then increased in *A. ervi* emerged from hosts older at parasitization. Body size values as related to host age at parasitization were achieved by adjusting developmental time, developmental rate or both. Parasitoids of similar size, but developed in hosts parasitized at different ages, had different wing cellular composition, while the increase of parasitoid body size was related to a general increase in both cell area and cell number. These results seem to suggest a trade-off between adult size and developmental time, at least for parasitoids developed at the two extremes of host ages at parasitization, and that *A. ervi* can reach the same adult size via different trajectories, adapting its ontogenetic processes. Wing shape was typical for all the different parasitoid classes considered and differed strongly between males and females, independent of their size. Parasitoid males (haploids) and females (diploids) did not differ in either cell area or cell number, suggesting a possible sex-determined dosage compensation in somatic tissue endoreplication. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **113**, 439–454.

ADDITIONAL KEYWORDS: cell number – cell size – developmental time – parasitoid – wing shape – wing size.

INTRODUCTION

Life history traits are phenotypic traits affecting life history, i.e. all the events that characterize the whole life cycle of an organism (Peters, 1983). Both life history and life history traits are related to fitness (Kojima & Kimura, 2003) and therefore reflect, at least in part, the relationship between an organism and its environment. Life history traits, such as developmental time, survival, fecundity, sex ratio and size, are usually used to evaluate potential activity of beneficial arthropods in natural and biological control of pests (Godfray, 1994; Roitberg, Boivin & Vet, 2001).

Life history traits are also used in quality control assessment of mass-reared arthropods (van Lenteren *et al.*, 2003).

One particular important life history trait is body size. Some life history traits, such as metabolic rate, energy requirements and reproductive success, are directly affected by body size and these traits may be predicted from a measure of size (Schmidt-Nielsen, 1983; Calder, 1984; Eijs & van Alphen, 1999; Luck & Forster, 2003). Body size has a strong effect on individual fitness (Roff, 1992). It has been shown that there is a strong correlation between body size and traits under selection and some authors discussed body size as a direct target of natural selection (Nagel & Schluter, 1998; Bonnet *et al.*, 2000; Reeve, Fowler & Partridge, 2000; Hayes & Shonkwiler,

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2006). However, we cannot expect that 'bigger' is always 'better'. In life history theory, the basic idea of optimization assumes that a balance of cost and benefits determines the value of a trait within the range of available variation, so the final size of an organism is optimized, not maximized (Stearns, 1992). Obviously, there are costs associated with a larger body size that decrease overall fitness: an increased juvenile mortality and a prolonged developmental time, which increases the chance of offspring predation (Sequeira & Mackauer, 1992a; Harvey, Harvey & Thompson, 1994).

In insects, adult body size is influenced by numerous physiological factors such as growth rates, duration of juvenile growth, and rate and duration of cell proliferation (Nijhout, 2003; Emlen & Allen, 2004; Edgar, 2006). Developmental and physiological processes influencing the growth and final size of adult body parts in insects have been mainly studied in *Drosophila* and to a lesser extent in some lepidopteran species (Emlen & Allen, 2004 and references therein). The definition of body (or organ) size and shape is a process that requires tight coordination of different cell dynamics, such as cell proliferation, apoptosis, cell allocation and mitotic orientation (Baena-López, Baonza & García-Bellido, 2005; Dworkin & Gibson, 2006).

In holometabolous insects, larvae are very different from adults and specialized for feeding and growing (Wake & Hall, 1999; Truman & Riddiford, 2002) but cells of imaginal discs, the origins of adult structures, proliferate during larval life. Growth of imaginal discs occurs primarily by cell proliferation (Emlen & Allen, 2004 and references therein). Each of the different imaginal discs behaves as a relatively autonomous developmental unit and the resulting dimensions of morphological structures will depend on how fast the cells in each imaginal disc proliferate and how long imaginal cell proliferation continues. The relative autonomy of the development of the different imaginal discs determines the allometries, namely the scaling relationships of body parts with body size, although the processes that coordinate final trait sizes with overall body size are little known (Emlen & Allen, 2004). The allometries have many ecological and adaptive implications and serve as important comparative tools in the systematics of insect taxa (Stern & Emlen, 1999; Nijhout, 2003; Shingleton *et al*., 2007).

In *Drosophila*, as well as in many insects, genetically homogeneous population produces individuals of different sizes and shapes depending on the environmental (external or internal) conditions (Trotta *et al*., 2010, 2011). It will be interesting to understand how environmental differences are translated, during development, into phenotypic differences. In insects, two particularly important environmental and ecological variables induce plasticity in body size: diet quantity/quality and temperature (Davidowitz, D'Amico & Nijhout, 2004; Trotta *et al*., 2006, 2010; Stillwell *et al*., 2007).

The wing of some insects (e.g. *Drosophila*) is regarded as an excellent model system to investigate size and shape variation. Wing area is positively correlated with body size as a whole and is considerably easier a feature to measure accurately (Robertson & Reeve, 1952; Partridge *et al*., 1999; Huey *et al*., 2000; David *et al*., 2005, 2006; Trotta *et al*., 2005a, 2011). Wing length was also used as a measure of body size for 40 species in 13 families of parasitoid wasps (Jervis, Ferns & Heimpel, 2003).

In *Drosophila*, the adult wing blade is produced by very flattened epidermal cells, and it has been proposed that cuticular trichome density gives an estimate of wing cell area that may reflect cell size in other body regions (Stevenson, Hill & Bryant, 1995; Partridge *et al*., 1999; Santos *et al*., 2005; Trotta *et al*., 2007). Forewing trichome density was also used as an estimator of cell density in ichneumonid parasitoid wasps of the genus *Diadegma* (Butcher, Whitfield & Hubbard, 2000a, b). As the ancestral pattern of insect imaginal disc development (which implies that cells commence proliferation near the end of the final larval instar) appears to occur in all of the basal holometabolous orders of insects (e.g. Megaloptera, Neuroptera and Mecoptera), and in the more basal families of the Coleoptera, Diptera and Hymenoptera, this estimator of the cellular component of body size can be extended to many insects (Truman & Riddiford, 1999, 2002).

Body size changes can be achieved through changes in cell size, cell number or both, and it has been shown that there is a strict relationship between cell parameter variation and fitness (Trotta *et al*., 2007). Among arthropods, *Drosophila* species and *Allonemobius fasciatus*, the striped ground cricket, were examined with regard to the relative contribution of cell size and cell number to body size (Arendt, 2007).

The wing vein network of most insects is regarded as an excellent model system for statistical analysis of variation in size and shape (Rohlf & Slice, 1990; Bookstein, 1991, 1996; Rohlf & Marcus, 1993; Dryden & Mardia, 1998; Klingenberg, Barluenga & Meyer, 2002). Wing size and shape can be analysed using geometric morphometric approaches, which precisely separate morphological variation (i.e. variation in form) into size and shape, which can be evaluated by using the Procrustes method to obtain coordinates of shape by removing the effects of size (Klingenberg *et al*., 2002). Geometric morphometrics approaches have been also used for the objective evaluation of morphological characters in a taxonomic context

(Baylac, Villemant & Simbolotti, 2003; Villemant, Simbolotti & Kenis, 2007).

In many parasitoids, adult size is a function of host size (Nicol & Mackauer, 1999). Host–parasitoid interactions show a wide range of adaptive strategies. Idiobiont parasitoids suppress the development of their hosts, so the colonized host represents a static food source and parasitoid adult size is strictly related to host size at parasitization. In contrast, koinobiont parasitoids allow their hosts to survive, and so they are able to parasitize the host before it attains the suitable size required for proper development of their progeny (Vinson, Pennacchio & Consoli, 2001; Pennacchio & Strand, 2006). In koinobiont parasitoids, a few studies on allometries as a function of host age/size are available (for *A. ervi* see Sequeira & Mackauer, 1992a, b), and the relative contribution of cell size and number to body size has not been approached.

Aphidius ervi Haliday (Hymenoptera: Braconidae) is an aphid parasitoid widely used as a model species in behavioural, physiological and molecular studies (Pennacchio, 1990; Battaglia *et al.*, 1993, 1995, 2000; Pennacchio *et al.*, 1999; Digilio *et al.*, 2000; Falabella, Tremblay & Pennacchio, 2000; Larocca *et al.*, 2005, 2007; Falabella *et al.*, 2007).

Like other koinobiont parasitoids, *A. ervi* may oviposit into any aphid instar (Pennacchio & Digilio, 1990); nevertheless, during the development to the proper size, aphid physiology, behaviour and reproduction are finely regulated to meet nutritional and physiological requirements of parasitoid larvae (Digilio *et al.*, 2000; Falabella *et al.*, 2000; Rahbé *et al.*, 2002; Pennacchio & Strand, 2006). Although any host stage may be accepted, the relationship between the host characteristics at oviposition and parasitoid fitness is not obvious (Colinet *et al.*, 2005). Different instars, even though all accepted as host, are not equivalent for parasitoid fitness, and the largest hosts may not necessarily be the optimal ones (Nicol & Mackauer, 1999; Chau & Mackauer, 2001; Colinet *et al.*, 2005; Henry, Gillespie & Roitberg, 2005).

In this study, phenotypic responses (plasticity) of developmental time, wing size and shape have been evaluated in *A. ervi* developed in *Acyrthosiphon pisum* (Harris) aphids parasitized at different ages. Our aim was to understand if *A. ervi* can reach the final adult size via different trajectories, adapting its ontogenetic processes to host age, and if wing size and shape are modulated in an independent way or not.

The parasitoid wing was used as an estimator of both *A. ervi* body size and its cellular composition as it is possible that the mechanisms contributing to body variation through changes in cell parameters are involved in the adaptation. We tried to under-stand if and how cell size and cell number contribute to an ecological variation in body size.

We observed how aspects in *A. ervi* wing development were related to external (different host size) and/or internal (haplo-diploid sex determination) environment, in terms of both different wing size/shape and cell parameters contributing to this variation.

MATERIALS AND METHODS

INSECTS AND EXPERIMENTAL PROCEDURE

The aphid, *Acyrthosiphon pisum*, was collected during spring from alfalfa (*Medicago sativa*) near Salerno, Italy (40°37′N, 15°3′E) and laboratory reared on broad bean plants (*Vicia faba* L). Aphid culture started in 1985 with a few hundred specimens. *Aphidius ervi* parasitoids were obtained from Koppert Italia and were laboratory reared on *A. pisum*. Aphid and parasitoid cultures were maintained in two separate environmental chambers at $20 \pm 1 \, ^\circ\text{C}$ and $75 \pm 5\%$ relative humidity (RH), under an 18:6-h light/dark (L/D) photoperiod.

To obtain synchronized cohorts of aphids, 40 apterous adult females were placed on a broad been plant and allowed to reproduce. After 24 h, the adult females were removed from the plant. The cohort of newborn nymphs was maintained as a synchronous colony on a broad bean plant. Two replicates of seven cohorts of different ages were thus obtained, from newly born to adult (1, 2, 3, 4, 5, 6 and 7 days old). Experiments were conducted in a climate-controlled chamber (21 °C, 60% RH, 16:8-h L/D).

The parasitoid females used were between 24 and 48 h old. Before the experiment, each female was left for 24 h with two males and fed on water and honey. Host parasitization was obtained by releasing one parasitoid female for 24 h on a broad been plant containing about 200 aphids of a given age. Based on previous observations, this number of aphids largely exceeds the capacity of a female parasitizing for 24 h and, in our experimental conditions, the superparasitism rate is negligible (less than 1%). Thereafter, the parasitoid female was removed and the aphids were maintained on a plant to continue their development until possible mummification occurred.

As a result of this experimental design, two replicates of seven experimental groups of parasitoids were obtained at emergence, i.e. from aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old, for a total of 318 parasitoid adults emerged.

DEVELOPMENTAL TIME

Developmental time was measured as the days elapsed between host parasitization and adult parasitoid
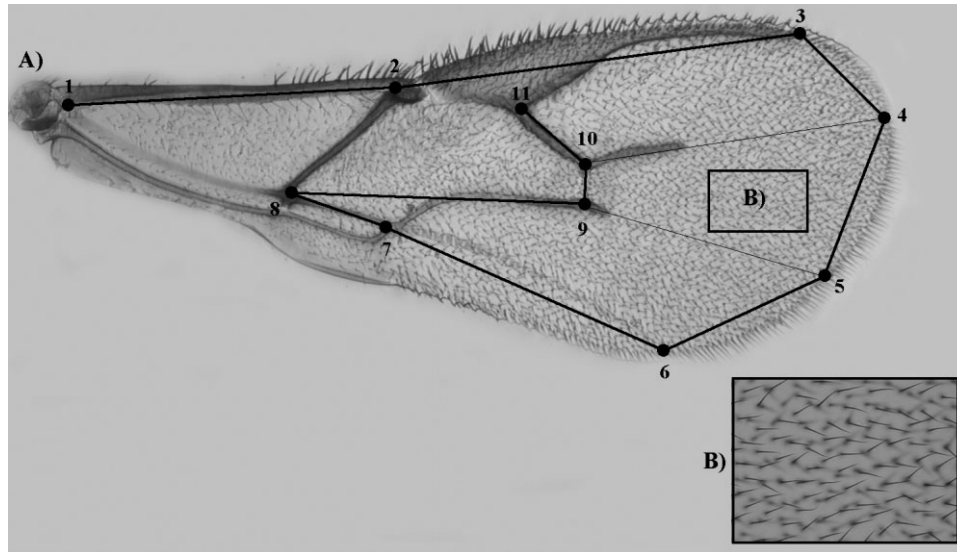
Figure 1. *A. ervi* wing. A, wing landmarks (1–11) used to analyse size and shape. The box (B) indicates the standard region used for trichome counting to estimate average cell area. On wings of different sizes, the region was chosen corresponding to the equivalent location with respect to veins and wing margin.

emergence. Emerged parasitoids were collected twice a day and their sex was determined.

WING SIZE, SHAPE AND ITS CELLULAR COMPONENT

Wing size and shape of both sexes from each experimental group were measured as previously described in Santos *et al*. (2004) and Trotta *et al*. (2011). Briefly, left wings were removed from each parasitoid and fixed under cover slips on microscope slides. Images of the wings were recorded with a Nikon video camera connected to a PC and mounted on a Nikon microscope.

The images were then used to record the *x* and *y* coordinates of 11 morphological landmarks (Fig. 1A). Using the original landmark coordinates, wing size was estimated as centroid size (CS) (Klingenberg & Zaklan, 2000; Debat *et al*., 2003; Santos *et al*., 2005; Trotta *et al*., 2011). CS is defined as the square root of the sum of squared distances of a set of landmarks from their geometric centre (the centroid) or, equivalently, the square root of the sum of the variances of the landmarks about that centroid in *x* and *y* directions (Slice *et al*., 1996).

After the landmark coordinates were recorded, all wing configurations were superimposed onto a consensus configuration (the overall mean configuration) using the Procrustes Generalized Least Square procedure (GLS, Rohlf & Slice, 1990; Bookstein, 1991; Rohlf & Marcus, 1993; Dryden & Mardia, 1998). Procrustes superimposition consists of three successive steps: (1) scaling: the configurations are scaled to

a unit centroid size; (2) translation: the centroid of each configuration is superimposed onto the centroid of the consensus configuration; (3) rotation: the configurations are rotated so as to minimize the distances between the corresponding landmarks, i.e. to optimize the superimposition (Dryden & Mardia, 1998). The new coordinates, or Procrustes coordinates, are amenable to standard multivariate analyses; as there are four eigenvalues that are zero in Procrustes fit, generalized inverses or Principal Component scores must be used.

To estimate the cellular components of body size differentiation, an image of the left wing was taken at 40×10 magnifications, and a sampling square of 0.0237 mm^2 was selected in the area of the distal part of the wing (Fig. 1B).

Trichome counting followed a standard protocol: the sampling area was visually inspected and the trichomes whose roots were within the selected square were marked with a black dot. Further manipulation provided a final image showing only the dots, which were counted using the Image J 1.31 software (http://rsb.info.nih.gov/ij/). Cell area was then estimated as 0.0237 mm^2 per dot. Because cell area is variable across the wing blade, a total cell number index was estimated as centroid size/cell area (mm^2) (Santos *et al*., 2004; Trotta *et al*., 2007).

To assess if wing size is a reliable estimator of body size in *A. ervi*, we also tested if and how wing size is positively correlated with other morphological characters. Wing size, head width, thorax and tibia length of the hind leg were afterwards measured in a

subsample of 132 individuals belonging to the seven experimental groups. For each individual, head width (the maximal distance between the eyes), thorax length (estimated as the distance between the joining with the head and the first leg) and tibia length were measured using a micrometer mounted on a Nikon microscope; wing size was estimated as centroid size, as explained above.

STATISTICAL ANALYSES

Relative to the incomplete cross between some veins and margin in the parasitoid wing (landmarks 4, 5 and in part landmark 6 – even if these landmarks are associated with a change in trichome polarity), measurements must be replicated to distinguish true shape differences among individuals from measurement errors. In this study, the wing shape of a subsample of 30 randomly chosen individuals developed on 3-day-old aphids was measured a second time without knowledge of the first measure. To compare the measurement errors with the among-individual variability, a one-way ANOVA with 'repeated measure' as random effect was performed on the measures of the landmark coordinates of these 30 individuals after Procrustes transformation. The 'repeated measure' effect was not significant for all the x, y coordinates of the 11 landmarks and the expected mean square of this term was very close to zero, explaining 6.6% of the total variance only in one case (landmark 1, x coordinate). A generalized least-squares algorithm, used to place all trials in a common coordinate system, distributes landmark error randomly across the configuration, thereby minimizing the overall error by reducing the residual variation around imprecise landmarks and increasing the variation around highly precise landmarks. This phenomenon has been dubbed 'the Pinocchio effect' (Chapman, 1990). On these bases, the wing shape measurement errors could be considered negligible.

To test for differences in developmental time, wing centroid size, cell area and cell number among experimental groups, mixed model ANOVAs with 'aphid ages' (i.e. parasitoids developed from aphids 1, 2, 3, 4, 5, 6 and 7 days old at parasitization), 'sex', as well as their interaction, as fixed effects, and 'replicate' nested within 'sex' and within 'aphid ages' were used. Tukey *post-hoc* tests for multiple comparisons of means were also performed to detect significant differences among the treatments of significant factor in the ANOVAs.

We tested for wing shape variation among 'aphid age', 'sex', for the interaction 'aphid age by sex' and between 'replicate' nested within 'sex' and within 'aphid ages' by applying a multivariate analysis of variance (MANOVA) to the scores of a principal com-ponent analysis (PCA) performed on the Procrustes coordinates, similar to the previously described ANOVAs.

A discriminant analysis followed by a canonical variate analysis considering the combination of 'sex' and 'aphid age' as independent factor was also performed. This approach allows us to optimally visualize the relative position of the different groups of parasitoids in the multivariate statistical space by maximizing the among-group variation.

A PCA was performed to visualize patterns of global shape variation (i.e. relative warps analysis) among 'aphid ages' and sexes. The relative warps were visualized with thin-plate spline transformation grids of landmark positions.

The Procrustes superimposition and the relative warps analysis were performed using PAST, version 1.90 software (Hammer, Harper & Ryan, 2001); all the other analyses in the present study were performed with R.3.0.0 software (R Core Team, 2013).

RESULTS

CORRELATION BETWEEN WING AND BODY SIZE

To test if wing size was a reliable measure of body size, we tested the correlation between wing CS size and three other measures of insect size. Considering together the seven experimental parasitoid groups, wing CS was highly correlated with thorax length ($r_{120} = 0.91$), head width ($r_{115} = 0.85$) and tibia length ($r_{130} = 0.84$) ($P < 0.001$ in all cases, Fig. 2). We also tested the relationships between wing CS and the other three measures of body size according to host age at parasitization and parasitoid sex, using ANCOVAs with 'sex' and 'aphid ages' as main effects (see Supporting information, Table S1). Considering the relationship with wing size, strong significant differences among the intercepts of 'aphid ages' (pooling sexes) were found for the three measures of body size. The intercepts of 'sex' (pooling aphid ages) were statistically different only for head and tibia length. No significant differences in slopes were found, except for tibia length ($P < 0.05$). As the differences in the intercepts could be ascribed to developmental allometries, as in other insects, we conclude that wing size can be considered a reliable measure of body size also in the parasitoid *A. ervi* (see also Jervis *et al.*, 2003).

DEVELOPMENTAL TIME

Aphidius ervi developmental time was significantly related to host age at parasitization. In the overall experiment, the sex ratio of all emerged parasitoids was female biased and approached 3:1. The proportions of female offspring produced were 0.48, 0.58, 0.9, 0.67, 0.54, 0.78 and 0.71 for parasitoids developed,
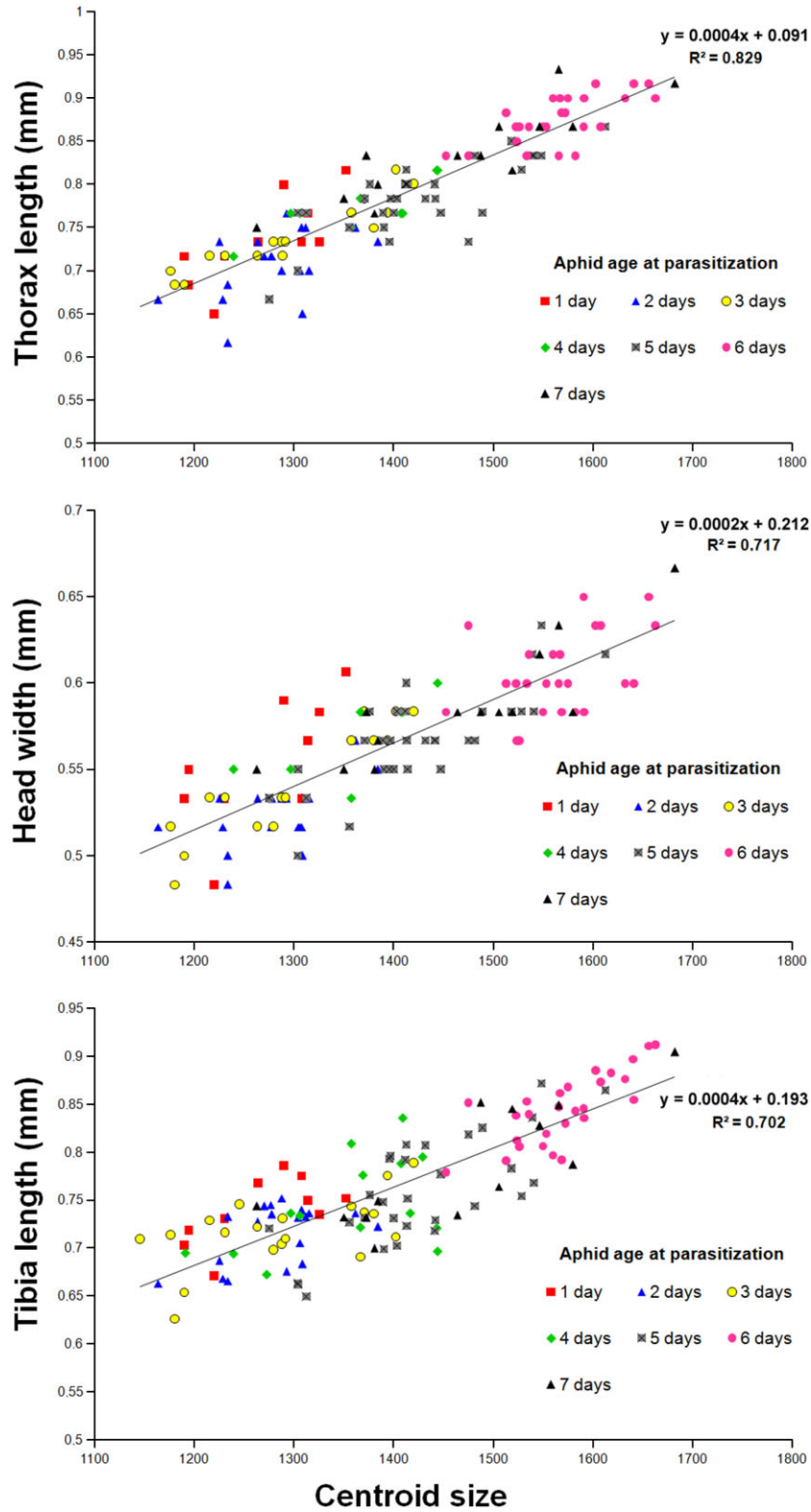
Figure 2. Relationship between wing and body size. Individual relationship between wing area (measured as centroid size) and thorax length, head width and tibia length in *A. ervi* developed in aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old.
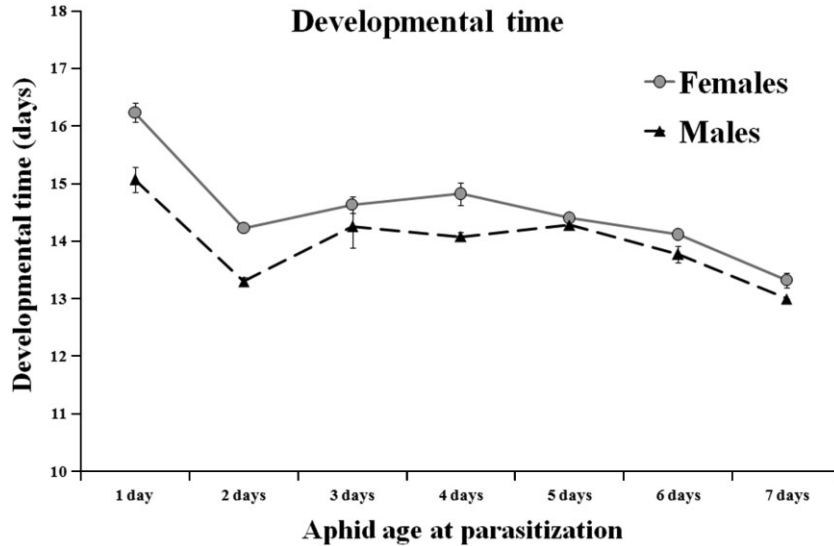
Figure 3. Developmental time differences. Mean developmental time in days (± standard error) of females and males of *A. ervi* developed in aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old. Sample size: 1 day, $N = 27$; 2 days, $N = 72$; 3 days, $N = 46$; 4 days, $N = 24$; 5 days, $N = 66$; 6 days, $N = 69$; 7 days, $N = 14$.

Table 1. Results of the mixed-model ANOVAs on developmental time, wing area, cell area and cell number of *A. ervi*

Source of variation	df	Developmental time		Wing size		Cell area		Cell number	
		MS	F	MS	F	MS	F	MS	F
Aphid age	6	14.7	42***	3.2×10^5	73***	1.7×10^{-8}	25***	1×10^6	30***
Sex	1	19	55***	3131	0.7	4.1×10^{-11}	0.8	5 483	0.153
Aphid age × sex	6	1.57	4.5***	2182	0.5	9.8×10^{-10}	1.4	23 916	0.66
Replicate within aphid age and sex	14	0.097	0.28	4174	0.95	7.8×10^{-10}	1.18	23 735	0.66
Residuals	290/290/263/263	0.346		4369		6.6×10^{-10}		35 793	

'Sex' and 'aphid ages' (i.e. parasitoids developed in aphids parasitized as 1, 2, 3, 4, 5, 6 and 7 days old) are fixed effects; 'replicate' is nested within 'sex' and 'aphid ages'.

***$P < 0.001$; *df*, degrees of freedom; MS, mean square; *F*, variance ratio.

respectively, from aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old. In this no-choice tests, offspring sex ratio differed in different host ages at parasitization ($\chi^2 = 27.9$, df = 6, $P = 9.6 \times 10^{-5}$).

Figure 3 shows the mean developmental times of the seven experimental groups of parasitoids obtained when developed from aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old. A mixed-model ANOVA (Table 1) gave significant differences as related to the aphid age at parasitization, between male and female parasitoids as well as a significant 'aphid ages by sex' interaction ($P < 0.001$ in all cases). No significant differences between replicates were found.

In both female and male parasitoids, mean developmental time was longer for parasitoids developed in aphids parasitized at 1 day old compared with the other aphid age classes (Tukey post-hoc test: $P < 0.001$ in all cases). In aphid hosts parasitized when 2–5 days old, *A. ervi* developmental times were not different, with a further time decrease in aphids 6 ($P < 0.05$ in all cases) and 7 days old at parasitization ($P < 0.001$ in all cases). In all the aphid ages at parasitization, we observed a significantly longer developmental time in females than in males ($P < 0.001$, Fig. 3).

WING SIZE

In the present study, wing size was used as a measure of body size on the assumptions that wing area is
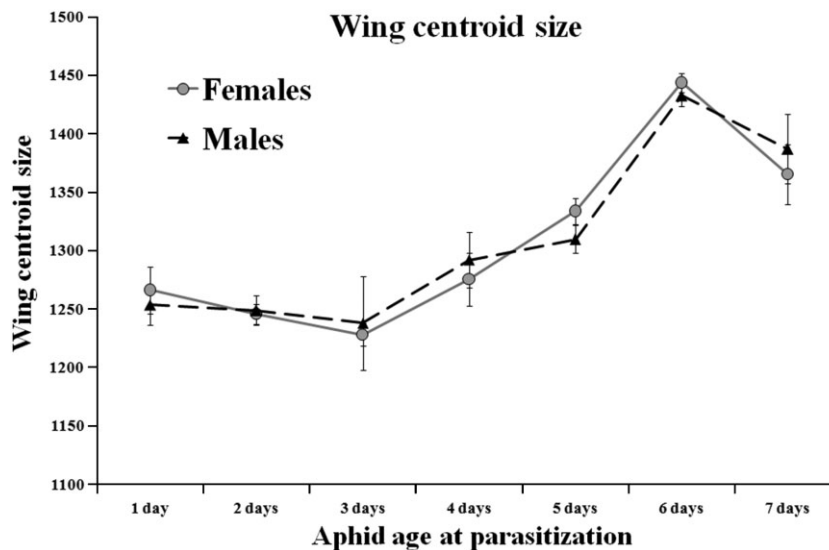
Figure 4. Centroid size differences. Mean centroid size (± standard error) of females and males of *A. ervi* developed in aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old.

positively correlated with body size (see Trotta *et al.*, 2011 and references therein) and that correlation estimates with other body size measures shown above are high. Mean values of wing CS of the seven experimental groups of parasitoids obtained from aphids parasitized at different ages are shown in Figure 4.

Significant differences in wing CS among the seven parasitoid groups were found ($P < 0.001$, Table 1). Interestingly, there is not a clear trend in the increase of parasitoid body size according to the increase of host age at parasitization: *A. ervi* developed in aphids parasitized as 1, 2 and 3 days old showed the same wing CS; size then increased when older aphids were parasitized (Tukey post-hoc test: $P < 0.001$ in all cases) and no further increase was observed when hosts were parasitized as 7 days old (Fig. 4). No significant differences between replicates, sexes or in the interaction 'aphid ages by sex' were found (Table 1).

No significant correlation between developmental time and wing size was found in both males and females ($r_5 = -0.48$ and $r_5 = -0.46$, respectively).

CELL AREA AND CELL NUMBER

The cellular components of body size differentiation showed a different pattern from that of wing size among the aphid age classes (Fig. 5). Mixed-model ANOVAs of cell area and cell number (Table 1) detected only significant differences among aphid age classes. No statistically significant differences

between replicates, sexes or the interaction 'aphid ages by sex' were found.

Parasitoid wing cell area was not significantly different in aphids parasitized as 1 and 2 days old, decreased significantly in aphids parasitized as 3 days old (Tukey post-hoc test: $P < 0.001$) and then increased in older aphid classes ($P < 0.001$). Cell number was significantly lower when aphids were parasitized as 1 and 2 days old, then increased in the other host classes ($P < 0.05$ in aphids parasitized as 1 versus 4 days old; $P < 0.001$ in all the remaining cases) but a strong decrease was observed in aphids parasitized as 7 days old (Fig. 5).

A principal component (PC) analysis for cell size/cell number clearly defined an inverse relationship between both variables (a general increase in cell area is followed by a decrease in cell number and vice versa). Component 1 (54% of the total variance) loadings are: cell area 0.77, cell number −0.24; Component 2 (46% of the total variance) loadings are: cell area −0.18, cell number 0.8.

Overall, the same wing size showed by parasitoids developed in aphids parasitized as 1, 2 and 3 days old was mainly obtained with a balance between number and area of cells. In parasitoids developed in aphids as 4, 5 and 6 days old, larger size was instead related to an increase in both cell area and cell number. Finally, compared with parasitoids developed from aphids parasitized when 6 days old, the size of the '7-day host' parasitoids was mainly the result of a balance between a strong decrease in the number of cells in the wing blade, and an increase in cell area (Fig. 5).
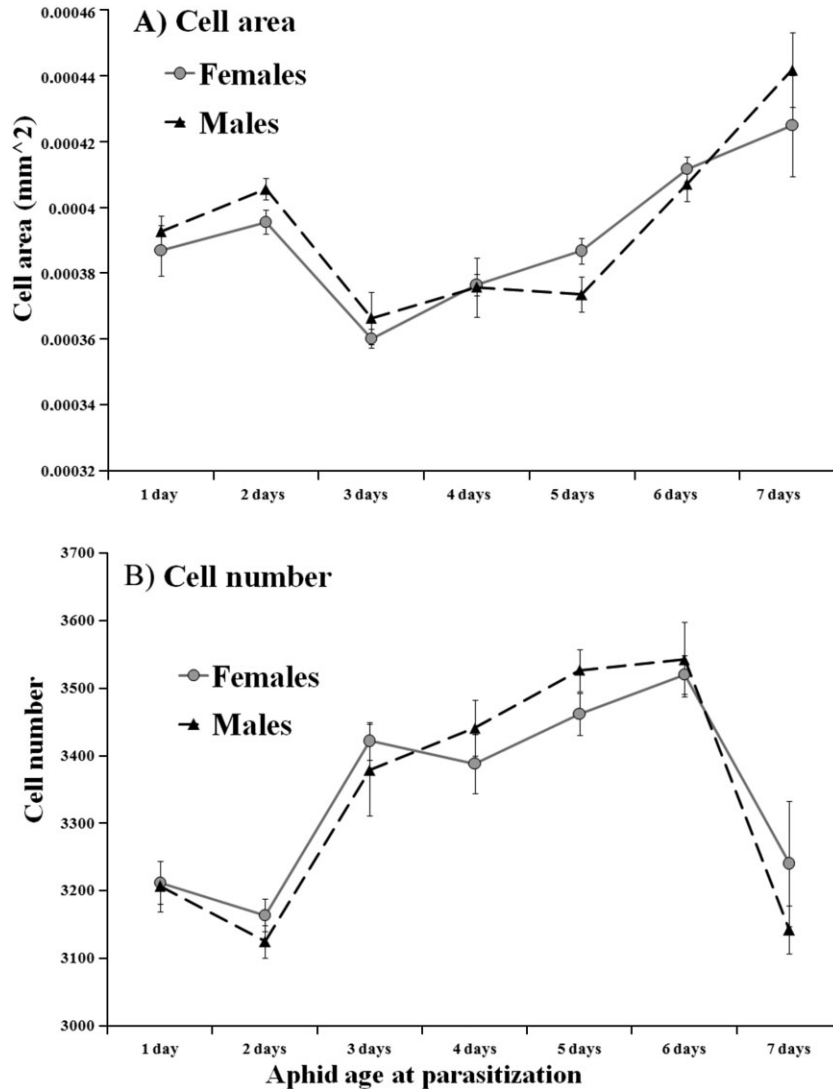
Figure 5. Cell area and cell number differences. Mean values (± standard error) of cell area (A) and cell number (B) of females and males of *A. ervi* developed in aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old.

WING SHAPE

A highly significant 'sex' effect was found in the MANOVA on Procrustes coordinates (Table 2), suggesting that wing shape varied strongly between female and male parasitoids, although the two sexes showed the same wing size. The between-replicates effect and the 'aphid age' effect were also significant and similar (Table 2), indicating that shape differences existed among parasitoids developed in aphids of a given age as well as of different ages and, consequently, of different size. The interaction between 'aphid age' and parasitoid sex was not significant.

The discriminant analysis combined with a canonical analysis provided clear discrimination between *A. ervi* females and males as well as among aphid ages (the first canonical axis accounted for 41% and the second for 28% of the total variance, see Fig. S1). As for developmental time, this indicates clear differences between sexes in wing shape, independent of wing size and its cellular components.

To test if the differences in wing shape were explained by differences in developmental time, a further MANOVA similar to the above described but using developmental time as a covariate was performed on Procrustes coordinates. The results were very similar to those reported in Table 2 (data not shown), indicating that the shape differences among sexes and 'aphid age' are independent of developmental time.

Table 2. Results of the MANOVA with 'aphid age' and 'sex' as fixed effects and 'replicate' nested within 'sex' and 'aphid ages' performed on the PC scores of the Procrustes coordinates of *A. ervi* wings

Source of variation	df	Pillai	Approx F	Num df	Den df	Pr(> F)
Aphid age	6	0.58	1.56	114	1662	0.0002
Sex	1	0.19	3.3	19	272	7.8×10^{-6}
Aphid age × sex	6	0.45	1.18	114	1662	0.103
Replicate within aphid age and sex	14	1.12	1.3	266	3990	0.0009
Residuals	290					

df, degrees of freedom; Pillai, Pillai's trace: one multivariate criteria test statistics used in MANOVA, calculated using the generated eigenvalues; *F*, variance ratio.

SHAPE RECONSTRUCTION

The shape changes (viewed as thin-plate spline deformation grids) for females and males parasitoids are shown in Figure 6. The mean shape is taken as the reference. The figure shows the area expansion (or contraction) around each landmark (computed using the Jacobian of the warp). These 'expansion factors' are colour-coded for all grid elements, with red for expansion and blue for contraction. In each given host class the differences between male and female groups are evident.

DISCUSSION

Nutrient availability is one of the major cues influencing growth, through the control of insulin and other growth factors, both sensitive to larval nutrition (Weinkove *et al*., 1999; Day & Lawrence, 2000; Johnston & Gallant, 2002; Nijhout, 2003; Edgar, 2006). In the case of parasitoids, nutrient availability depends on many variables including host size. Parasitoids distribute their eggs on resources present in discrete units (host body) and then confine their offspring to a more or less fixed amount of food. Parasitoid larvae have no availability of other food resources than their original host to complete their development. For this reason, behaviour and physiology of parasitoids is under a strong selective pressure. In koinobiotic species, such as *A. ervi*, parasitoid larvae feed while hosts continue their development. The host continues to grow and moult normally while the larval parasitoid reaches a critical size. The maximum larval dry mass, time from oviposition to adult eclosion, and final size of parasitoids vary with host age at parasitization (Sequeira & Mackauer, 1992a, b). So the parasitoid exhibits a phenotypic plasticity of body size depending on host age, with a non-linear relationship between body size and host age. Some of our results on body size, development time and allometric relationships between size of males and females in relation to host age seem somehow different from some of the data reported by

Sequeira & Mackauer (1992b). This can be ascribed partially to the fact that they estimated size in terms of dry mass and there is not necessarily a linear relationship between dry mass and dimensions of insect body or parts of it. Other differences, in particular those relating to the relationships between host age and development time, may depend on the aphids rearing conditions (24 h of light in Sequeira & Mackauer, 1992b) and/or on the measure of developmental time (estimated continuously in Sequeira & Mackauer, 1992b). Moreover, Sequeira & Mackauer (1992b) produced four experimental aphid ages (22, 46, 70 and 118 h) instead of our seven host ages. As a result, the aphid life span we tested as hosts is broader and we added an earlier host age and at least a final one that were not tested by Sequeira & Mackauer (1992b). All this considered, we think that our results do not substantially disagree with those reported by Sequeira & Mackauer (1992b). Using wing size as an estimator, we confirmed the phenotypic plasticity of body size in *A. ervi*, with the final size depending on host age, but with a non-linear relationship between body size and host age.

From an ontogeny-focused point of view, the difference in body size (or the equality) can be achieved by varying developmental time (keeping parasitoid developmental rate constant), by varying parasitoid developmental rate (keeping developmental time constant) or by varying both of them. In our work, parasitoids developed in hosts 1, 2 or 3 days old at parasitization resulted in the same size, both in males and in females (Fig. 4). This result required a longer developmental time in hosts parasitized when 1 day old, compared with the other two ages (Fig. 3). We do not know if parasitoids emerged from aphids 1 day old at parasitization changed their growth rate or if the parasitoid larvae slow down their development until the host has reached a sufficient size (Hu, Gelman & Blackburn, 2003) because the aphids were not dissected. However, from an evolutionary perspective, the same size shown by parasitoids emerged from aphids 1, 2 or 3 days old at parasitization could
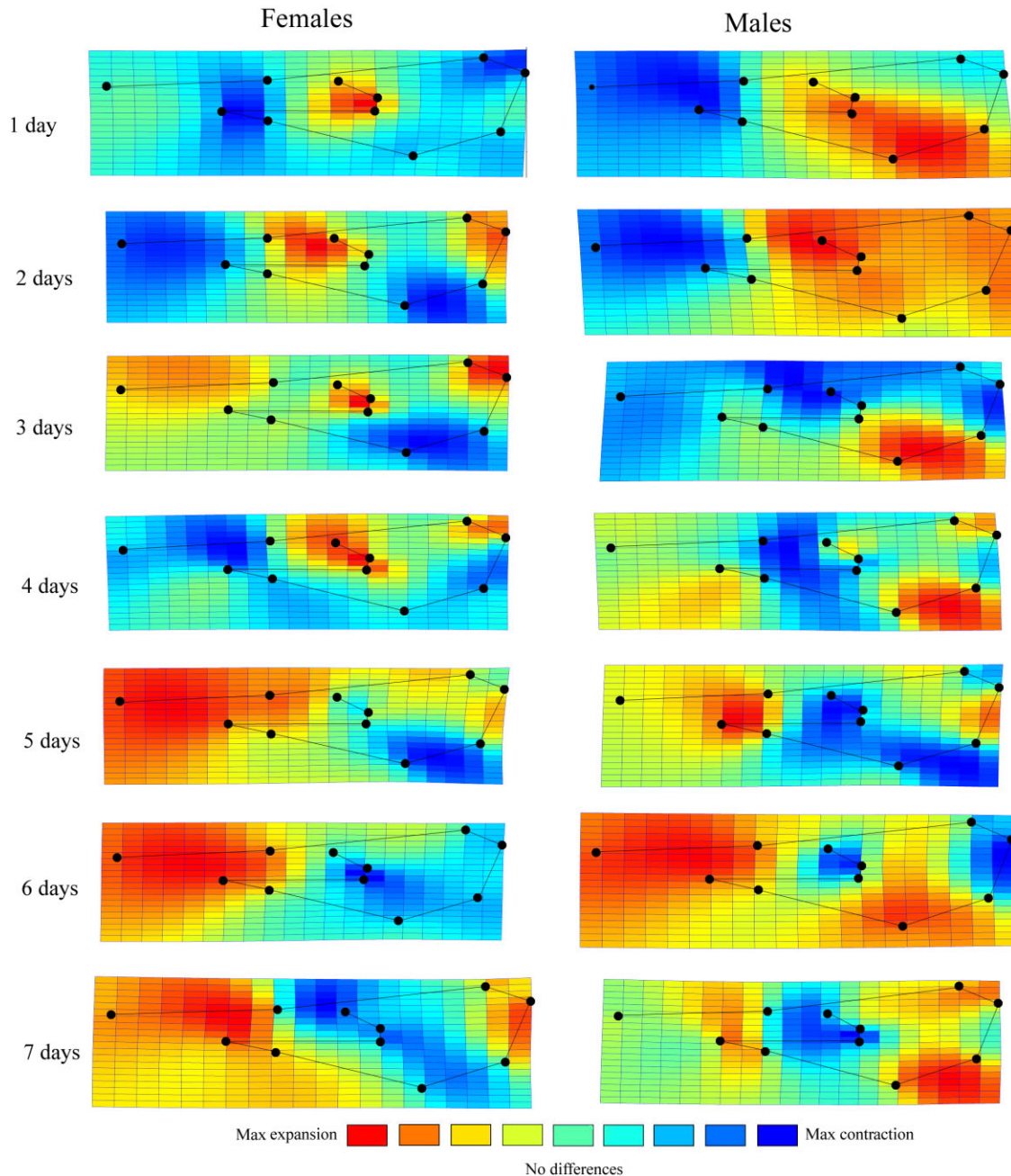
Females   Males

Figure 6. Thin-plate spline deformations. Wing shape differences between females and males of *A. ervi* emerged from aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old are shown as thin-plate spline deformations. Deformation grids show the shape changes (yellow to red for expansions and light to dark blue for contraction) relative to a mean shape.

be explained if parasitoids are directly selected to reach a minimal size (or a 'minimum viable weight', Edgar, 2006) that allows larvae to survive to adult and ensures adults have an appropriate fitness under specific environmental situations. In aphids parasitized when 4, 5 and 6 days old we observed an increase in size that is related to a greater developmental rate, as the developmental time is substan-

tially unchanged. A possible explanation could be than being older and so larger, a host provides greater and/or better feeding resources to the developing parasitoid than smaller hosts, but this cannot be the only explanatory factor because we observed an arrest of the size increase when the aphid was parasitized as 7 days old. Adult aphids have a lower life expectancy and the probability that the host dies before the

parasitoid has completed its development increases. Consequently our data seem to suggest that, to maximize fitness, parasitoids emerged from adult aphids at parasitization have been selected for a reduction in their developmental time that resulted in a smaller size, albeit not significant, than the previous host age.

Taken together, these results suggest that there is a trade-off between adult size and developmental time at least for parasitoids developed in the two extreme classes of host size (i.e. 1- and 7-day-old aphids at parasitization). The selective pressures operating on body size and on developmental time could be different in direction and intensity: if a reduced developmental time were at a premium, an appropriate body size that increases the overall fitness should be reached.

Note that, within a given host age at parasitization, males and females of *A. ervi* had the same size, although they showed low but significant differences in developmental time.

What is more interesting is the variation in cellular components of parasitoid wings. This is the first time that the cellular components of body size of *A. ervi* have been investigated as a fundamental part of the relationships between parasitoid size and host size. Our results suggest that *A. ervi* can reach the same adult size via different trajectories and can adapt its ontogenetic processes to host age.

As stated above, parasitoids emerged from hosts 1, 2 and 3 days old at parasitization are similar in size but they have different cellular composition, reflecting the environmental conditions during their development. These three parasitoid classes showed pronounced differences in cell number followed by a sort of compensation in cell area (Fig. 4). By contrast, the bigger sizes of adults emerged from aphids 4, 5 and 6 days old at parasitization are due to a general increase in both cell area and cell number. The cellular components of parasitoids emerged from aphids 7 days old at parasitization clearly showed the presence of cellular compensatory mechanisms between the cell area and cell number, at least in the wing blade. The final result was that no strong size reduction was observed in these parasitoids. It is reasonable to suppose that those cellular compensatory mechanisms are not only the outcome of a developmental buffering (Trotta *et al*., 2005b), but are also involved in adaptation as they ensure an increase in fitness by reaching an appropriate size.

Cell area and cell number must be considered if we try to understand regulation of body size, as different environmental factors (internal or external) influence body size through different mechanisms (Arendt, 2007). The size of adult body parts will depend on how fast the cells in each imaginal disc proliferate, and how long imaginal cell proliferation continues (Emlen

& Allen, 2004). Growth of imaginal discs occurs primarily by cell proliferation but changes in cell size may also influence trait sizes (Conlon & Raff, 1999; Montagne *et al*., 1999; Verdu *et al*., 1999; Weinkove *et al*., 1999; Johnston & Gallant, 2002). In *Drosophila*, genetic differences among populations or nutrient levels influence body size mainly through changes in cell number while cell area variation is more sensitive to other environmental variables such as temperature (Arendt, 2007; Trotta *et al*., 2007 and references therein).

On the basis of our results, some interesting considerations can be inferred: (i) the same size of adult parasitoids emerged from the 1-, 2- and 3-day-old host classes reflects different and independent developmental mechanisms influenced by both developmental time and developmental rate, with variation in cell area counterbalanced by variation in cell number; (ii) when parasitoids develop in bigger (and then more suitable) hosts, the greater parasitoid size is due to an increase of developmental rate reflected in an increase of both cell area and cell number; (iii) when parasitoids developed in hosts of the 7-day-old class cell proliferation is reduced (reduction also supported by a shorter developmental time) and the presence of cellular compensatory mechanisms leads to a relatively large final size.

For a given cell type, cell size is usually proportional to ploidy and haploid cells are about half the volume of diploid cells (Day & Lawrence, 2000). Instead, in *Drosophila* the growth and final size of tissues composed of diploid/haploid mosaics are similar to the wild-type, i.e. are not affected by the diploid/haploid mosaics (Santamaria, 1983), but the haploid regions of such flies contain a higher number of smaller cells. Our data show no differences between parasitoid males (haploids) and females (diploids) either in cell area or in cell number, perhaps suggesting a sex-determined dosage compensation in somatic tissue endoreplication. These results confirm what has been previously found in some Ichneumonoidea species, where mass or wing hair cell size were considered unreliable haploid–diploid discriminators at the individual level (Butcher *et al*., 2000b).

A final consideration concerns the parasitoid wing shape. Wing size and shape could be modulated independently, even if the basic developmental mechanisms underlying the morphogenetic process appear to be constrained (Trotta *et al*., 2011). As we showed that a similar wing size could be achieved through different developmental mechanisms, it is important to gain more knowledge on the different components that govern variation in morphology. The results of the present study show that wing shape is typical for all the different parasitoid classes considered and, more importantly, wing shape is strongly different for

males and females, even if they have the same size. The independence of size and shape allows a certain level of flexibility in evolutionary adaptation of wing shape, irrespective of changes in size but associated with the evolution of developmental time and then with local mate competition, where males compete for mates in small colonies and females disperse following mating (Hamilton, 1967; Charnov, 1982). More rapid development of male parasitoids is favoured as they may be able to mate with more females than later eclosing males (Godfray, 1994; Harvey & Strand, 2003).

In *A. ervi*, within a given host age at parasitization, large females do not benefit more in terms of fitness than large males as no sex dimorphism was observed; by contrast, selection for reduced developmental time may be more important in males than in females, leading to dimorphism for this trait. At present, the causes of variation in wing shape and in developmental time between males and females remain unknown, as well as the possible relationships between these two traits, particularly if we consider that size and its cellular component remain unchanged between sexes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Wing shape variation. Mean values (± SE) of the first and second canonical plans computed from the canonical variate analysis on the PC scores of the Procrustes coordinates (shape variation) of females and males of *A. ervi* developed in aphids parasitized when 1, 2, 3, 4, 5, 6 and 7 days old.

Table S1. Results of the ANCOVAs on the relationship between wing area (measured as centroid size) and, respectively, thorax length, head width and tibia length in *Aphidius ervi*. 'Sex' and 'aphid ages' (i.e. parasitoids developed in aphids parasitized as 1, 2, 3, 4, 5, 6 and 7 days old) are fixed effects.