



## Transgenerational plasticity in aphids reared in a poor-resource environment

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### ABSTRACT

The changing environmental conditions can affect insect biology over multiple generations and phenotypic plasticity is important for coping with these changes. Transgenerational plasticity occurs when the environment in which the parents developed influences the plastic response of the offspring phenotype. In the present study, the plastic effects of resource limitation on important life history traits such as body size, fecundity, survival, and resistance to starvation of the pea aphid *Acyrtosiphon pisum* were investigated over two generations. This study focused on understanding how resource limitation can determine an adaptive expression of maternal effects and transgenerational plasticity in fitness-related traits. Aphids showed phenotypic plasticity for the life history traits investigated, as they performed better when grown in an optimal environment than in a resource-poor one. Also, aphids had a poorer performance if their mothers were raised in a resource-poor environment. The effects of transgenerational plasticity were observed only in response to resistance to starvation, through increased survival in the offspring of the mother reared in a resource-poor environment, suggesting an evolutionary bet-hedging strategy. The results of this study showed that the effects of adaptive transgenerational plasticity may be partially masked in stressful environments, where developmental problems instead predominate. More information on the transgenerational response to resource limitation across generations can contribute to a better understanding of aphid biology.

### 1. Introduction

The changing environmental conditions to which living organisms are exposed can affect their phenotype and, consequently, their fitness over multiple generations (Hoffmann and Parsons, 1997). Phenotypic plasticity (the ability of a single genotype to produce different phenotypes in response to changes in environmental conditions) is considered particularly important for coping with environmental variation (West-Eberhard, 2005). Plasticity responses can be reversible or irreversible, adaptive or non-adaptive. An irreversible plastic response means that the expression of a particular phenotype is determined during development and remains unchanged throughout the life of an organism, whereas a reversible phenotypic response means that a certain trait can be reversed to the original state (for example, changes in behavior or some physiological traits).

Plasticity can be considered adaptive if a particular developmental

variant produced under certain conditions has a positive effect on fitness (Pigliucci, 2001; Arnold et al., 2019; Levis and Pfennig, 2016). As a result of phenotypic plasticity, individuals can rapidly change their phenotype in response to their surrounding environment. Phenotypic plasticity can also evolve; for example, in *Drosophila melanogaster* Meigen, the phenotypic plasticity of some life history traits is adaptively modified as a result of colonization of temperate continents (Trotta et al., 2006).

Maternal effects occur when the phenotype of an individual is influenced across generations by the environment experienced by its mother (Mousseau and Dingle, 1991). Maternal effects can be considered as a mechanism of adaptive plasticity as mothers transmit information about environmental variability to their offspring, producing offspring that are adapted to the conditions they may encounter (Mousseau and Fox, 1998). The influence of the maternal environment appears to be more important for offspring reared under stressful

**Abbreviations:** TGP, Transgenerational plasticity; F<sub>0</sub>, parental generation; F<sub>1</sub>, offspring generation; O, optimal resource scenario; P, poor-resource scenario; RH, relative humidity; L:D, Light:Dark photoperiod; GLM, generalized linear models; LMM, linear mixed-effects model; REML, restricted maximum likelihood.

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conditions. For example, in the bryozoan *Bugula neritina* (L.), mothers reared under high levels of crowding produced offspring with a high ability to escape crowding (Allen et al., 2008), confirming that mothers can adaptively adjust the phenotype of their offspring under stressful conditions.

Transgenerational plasticity (TGP) is the term used to describe a particular type of plasticity and maternal effects in which the current environmental conditions of the parents influence the offspring generations in a non-genetic inheritance manner through variations in their phenotypic plasticity (Badyaev and Uller, 2009; Burgess and Marshall, 2014; Salinas et al., 2013; Yin et al., 2022). TGP is a type of intergenerational inheritance that can only be measured when there is environmental variation in both the parental and offspring generations. Transgenerational responses could be adaptive for offspring if they show phenotypic variation across environments, with increased resistance to certain stressful conditions (Agrawal et al., 1999). TGP has been observed in many arthropods in response to various stresses such as heat (Diaz et al., 2021; Ledón-Rettig 2023), drought (Le Hesran, 2020) and predation (Hermann et al., 2021; Mondor et al., 2005). TGP occurs when parents modify both the trait mean and slope of the offspring reaction norm, whereas in the presence of maternal effects alone, the offspring phenotype is indeed influenced by the mother, but no plasticity in response to a changing environment is observed. Maternal effects are useful for coping with changing environments when parents can effectively predict the environment of their offspring. However, when the environmental variance is unpredictable, TGP is very important because it allows the offspring to accurately use parental cues in a changing environment.

Aphids, insects that do not provide parental care, are important pests of many economic crops and are often used as model systems to study the causes and effects of TGP. This is due to their parthenogenic reproduction (unfertilized eggs develop into females) and to the telescoping generations (the mothers contain daughters, which in turn also contain embryos). Parthenogenic viviparous aphid females contain embryos that carry their own offspring (Kindlmann and Dixon, 1989), resulting in overlapping generations exposed to a given environment. It has been hypothesized that aphids have evolved genetic mechanisms capable of conferring transgenerational plasticity mediated by transposition, a specific form of genetic recombination mediated by repeat units of transposons (De Fabrizio et al., 2024). The phenotype of the aphids is then influenced by both the maternal and the current environment in which they have developed (Mousseau and Dingle, 1991). For example, in crowded aphid colonies, apterous morphs can produce winged offspring that can quickly leave the colony and avoid plant deterioration (Braendle et al., 2006). Increased predation in the maternal environment also induces TGP in *Aphis gossypii* (Glover) and *Acyrtosiphon pisum* (Harris), with an increase in the number of winged offspring (Kunert and Weisser, 2003; Mondor et al., 2005).

Food quality and quantity are important factors influencing insect development and are highly unpredictable in natural environments. Under adverse conditions such as starvation (Scharf, 2016), adult *Sitobium avenae* (Fabricius) aphids showed reduced longevity and fecundity (Xu et al., 2019). Herbivorous insects exhibit a high degree of phenotypic and transgenerational plasticity in their physiological and behavioral responses to changes in host plant quality, which in turn affects how they interact with the plant (Ashra and Nair, 2022; Hayden et al., 2021). Important life history traits of aphids, such as body size, survival, developmental rate, and fecundity, are negatively affected by a poor quality of the host plant (Dixon, 1987; Hayden et al., 2021; Ríos Martínez and Costamagna, 2018) or when the plant is damaged by other herbivores (Denno et al., 2000; Takemoto et al., 2013). The feeding environment is then important in modulating the phenotype of aphids, as these organisms can alter their physiological and morphological traits to adapt to the new current environment. Aphids can transmit information about the current feeding environment to their offspring, which in turn can adjust their traits to improve fitness. However, it is unclear

whether the phenotypic response to resource variation in aphids is primarily influenced by current environmental factors, by maternal effects, or by their interaction. For example, body size and fecundity of *Aphis gossypii* are influenced by the nutritional quality of the host plants, but a stronger effect is due to the quality of the host plant on which the parental generation feeds (Nevo and Coll, 2001). In *Aphis nerii* (Fonscolombe), variation in some life history traits may depend on current and maternal resource availability, but there is no evidence for adaptive maternal effects (Hayden et al., 2021).

Because food resources can be unpredictable in natural environments, management of energy reserves is important for aphids to successfully survive periods of food deprivation. Starvation tolerance could be considered an important fitness-related trait that is subject to maternal effects (Gliwicz and Guisande, 1992), as many insects arrest or resorb oocytes to increase their tolerance to food deprivation (Bell and Bohm, 1975; Brough and Dixon, 1990; Xu et al., 2012). Offspring produced by mothers from higher quality environments are more likely to survive when adverse conditions are moderate or absent (Allen et al., 2008; Dixon, 1998), but if adaptive TGP mechanisms occur, offspring from lower quality maternal environments should be better adapted to certain severe stress conditions. Aphid responses to starvation may also vary depending on the simultaneous co-occurrence of different stressors, as has been documented for thermal stress (Trotta et al., 2018; Forlano et al., 2023).

Using the pea aphid *Acyrtosiphon pisum* as a model organism, this study investigates how aphids modulate the plastic response of some of their life history traits in a resource-limited environment such as cut broad bean leaves with the stem in tap water. *Acyrtosiphon pisum* can complete its development on a cut broad bean leaf but exhibits lower survival, smaller body size, and reduced fecundity, characteristics common to aphids that have been developed in a low nitrogen environment (Ponder et al., 2000; Wilkinson et al., 2007). To determine the plastic and transgenerational effects of food variation in the maternal and current environments, the resource availability was manipulated for two generations. The aim of this study was to determine the degree of plasticity, maternal effects, and TGP on survival, fecundity, body size, and tolerance to starvation of aphids born from mothers reared in normal or resource-poor environments and exposed to the same feeding conditions in the next generation. We investigated the extent to which resource scarcity might determine the expression of adaptive maternal effects and TGP in fitness-related traits. A poor maternal environment could constrain the life history traits of the next generation, as transgenerational effects may depend on current resources. We discuss the possibility that starvation tolerance may exhibit adaptive TGP, suggesting an evolutionary “bet-hedging” strategy (Cohen, 1966; Joschinski and Bonte, 2020; Slatkin, 1974) with a mix of fitness costs and gains that emerge over generations. Incorporating the effects of resource limitation across generations on plasticity, maternal effects, and transgenerational plasticity can contribute to a better understanding of aphid biology.

## 2. Materials and methods

### 2.1. Insect rearing

Broad bean plants, *Vicia faba* L. c.v. Agua dulce (Fabales: Fabaceae), were grown in pots (10 cm in diameter, 500 cc volume) with commercial soil (COMPO SANA® Universal Potting Soil) in a greenhouse. Broad bean plants with three or four pairs of leaves were used approximately 3 weeks after germination.

The pea aphid *Acyrtosiphon 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 for ten years. The pea aphid was reared in the laboratory at room temperature under an 18L:6D photoperiod. To eliminate maternal and grandmaternal effects from the initial experimental individuals, groups of 60 adult virginoparae females were isolated from the aphid culture, placed on a fresh host plant kept in a

plastic box (20 × 15 × 30 cm height), and allowed to reproduce for 24 h in a Binder KBF climatic chamber at 22 ± 1 °C, 75 ± 5 % relative humidity (RH) and an 18:6 Light:Dark (L:D) photoperiod. Adults were then removed and the cohort of newborn nymphs was reared to adulthood. To avoid any possible effect of host plant deterioration, 4-day-old aphids were transferred to a new plant with a soft brush for another 4 days. At the end of this 8-day period, the aphids were at the beginning of the adult stage. This procedure, carried out for 2 generations, was repeated independently for 7 groups of aphids, resulting in individuals from 7 independent replicates.

## 2.2. Experimental design

In this experiment, an optimal and a resource-poor environment were set up to simulate the effect that short-term fluctuations in host plant quality could have on aphid performance. For the optimal resource scenario (O), 30 first instar aphids were allowed to develop on a broad bean plant grown in a pot with soil for 8 days, that is, until they became adults. The poor-resource scenario (P) consists of three separate groups of 10 aphids developing on a pair of cut broad bean leaves, with the stem in an Eppendorf tube filled with tap water and sealed with parafilm to prevent desiccation. The leaves were placed in a plastic cylinder (diameter: 5.5 cm, volume: 150 ml) with a mesh-covered ventilation hole in the screw cap. Due to the deterioration of the leaves, after 4 days, the aphids were carefully transferred to a new pair of cut leaves for another 4 days, until they became adults.

A total of 100 adult apterous females of *A. pisum* (9–10 days old) were isolated from a plant and reared under the above standard conditions on a new potted broad bean plant in a climatic chamber. To ensure offspring of the same age, adult females were allowed to reproduce on a fresh plant for 24 h to obtain the F<sub>0</sub> newborn nymphs. Sixty first instar *A. pisum* nymphs of the F<sub>0</sub> generation were then divided into two groups and allowed to develop in the experimental environments O and P until they became adults. The two treatments, corresponding to the two maternal environments of the next generation, were coded as OF<sub>0</sub> and PF<sub>0</sub>, respectively.

When aphids from the OF<sub>0</sub> and PF<sub>0</sub> environments reached adulthood, their offspring (F<sub>1</sub>) were used in the following studies. The F<sub>0</sub> mothers were allowed to reproduce for 24 h in their own environment (the OF<sub>0</sub> aphids on a fresh plant and the PF<sub>0</sub> on 3 pairs of cut broad bean leaves). Sixty newborn first instar nymphs F<sub>1</sub> were obtained from OF<sub>0</sub> and PF<sub>0</sub> adult females. These aphids were then divided into two groups and allowed to develop to adulthood in the experimental environments O and P, resulting in four F<sub>1</sub> experimental treatments. Each treatment had 7 replicates and each replicate started with 30 newborn first instar nymphs. The four treatments were coded as OF<sub>0</sub>-OF<sub>1</sub>, OF<sub>0</sub>-PF<sub>1</sub>, PF<sub>0</sub>-OF<sub>1</sub>, and PF<sub>0</sub>-PF<sub>1</sub>; the first part of the code refers to the maternal environment, and the second part refers to the current environment. A schematic representation of the experimental procedure is reported in Figure S1.

## 2.3. Measurements

For all experiments, the aphids were reared and maintained in climatic chambers (22 ± 1 °C, 75 ± 5 % RH and 18L:6D). For the F<sub>0</sub> generation, measurements of survival (from nymph-to-adult and at day 14), fecundity (from adult to day 14), and body size were determined at day 14. For the F<sub>1</sub> generation, nymph-to-adult survival, adult body size, survival, fecundity at three-time intervals (between days 9–12, 13–16, and 17–20), and adult tolerance to starvation were recorded.

Fecundity during a given time interval was defined as the mean number of nymphs per adult per day. Survival was estimated as the number of living individuals relative to the initial number of aphids; an aphid was considered alive if it could walk or at least move when gently touched with a soft brush. Survival was first measured upon reaching adulthood (8 days after the start of the experiments); aphids were then

transferred to new rearing units (plants or leaves) every 4 days for three times, and fecundity and survival were measured at each transfer.

To estimate starvation tolerance, approximately 50 newly molted adults were randomly collected from the four F<sub>1</sub> treatments. Five replicates per treatment were used for this experiment, with each replicate consisting of an average of 9 individuals; unfortunately, due to the early deterioration of some plants, only 3 replicates were used for the OF<sub>0</sub>-OF<sub>1</sub> treatment. Starvation experiments were performed in Petri dishes with a water-soaked cotton wool to prevent desiccation effects. No food was provided to the aphids. Petri dishes were sealed with parafilm and kept at a constant temperature of 22 °C. Dishes were checked after 24 and 48 h and aphid survival was estimated as previously described.

Body size was estimated as the length of the adult hind tibia (Nicol and Mackauer, 1999; Trotta et al., 2014). The left hind tibia was removed from a sub-sample of approximately 60 individuals belonging to the experimental groups and fixed on slip-covered microscope slides. Images of the tibiae were recorded using a video camera connected to a PC and mounted on a Nikon microscope; length measurements were recorded using ImageJ software (Schneider et al., 2012). All measurements are in mm.

## 2.4. Statistical analysis

All data were analyzed using the statistical software R (R Core Team, 2022) with the packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017).

### 2.4.1. Effects of a food-restricted environment on life history traits of F<sub>0</sub>

To test the effect of the explanatory variable “treatment” (two levels, OF<sub>0</sub> and PF<sub>0</sub> environments) on nymph-to-adult survival (interval between days 0–8) and survival at day 14, two Generalised Linear Models (GLM) with binomial error (logit link function) were used, as a binomial distribution is appropriate for modeling binary data (dead / alive). Differences in fecundity of F<sub>0</sub> individuals were analyzed using a two-tailed *t*-test on a subsample of 6 replicates. Data on body size were analyzed using a linear mixed-effects model (LMM) fitted with REML (restricted maximum likelihood), with “treatment” as a fixed factor and “replicate nested in treatment” as a random effect. The *P*-values for differences between treatments were obtained by ANOVA (type II Wald chi-square tests). The assumptions of homoskedasticity and normality were checked and met for the productivity and body size data (Shapiro-Wilk tests).

### 2.4.2. Effects of food-restricted environment on F<sub>1</sub> life history traits

We tested whether the food-restricted environment applied to parental individuals also affected those of the next generation. The models for the analysis of the data from the F<sub>1</sub> generation included the two treatments “maternal environment” (two levels, OF<sub>0</sub> and PF<sub>0</sub>) and “current environment” (two levels, OF<sub>1</sub> and PF<sub>1</sub>) as fixed factors, together with their interactions. Survival and starvation tolerance of the F<sub>1</sub> aphids were analyzed using GLMs with binomial errors (logit link function). *P*-values for differences between “maternal” and “current” environments, and their interactions were obtained by analyses of deviance (type II Wald chi-squared tests). Differences in fecundity of the F<sub>1</sub> individuals were analyzed using two-way ANOVAs. Data on F<sub>1</sub> body size were analyzed using a linear mixed-effects model (LMM) fitted with REML, with “maternal” and “current” environments as fixed factors, and “replicate” nested within the fixed factors as random effect. The Shapiro-Wilk test was used to test the normality of the fecundity and body size data. For all statistical models performed, the two main effects and the interaction term were fitted for each data set. A backward procedure was used to sequentially remove non-significant effects, allowing the identification of the most parsimonious model.

### 3. Results

#### 3.1. Within-generational effects of a food-restricted environment on $F_0$ aphids

Development in a food-restricted environment had a strong effect on the life history traits of  $F_0$  aphids (Fig. 1). Compared to  $OF_0$  groups,  $PF_0$  aphids reared in a poor-resource environment had lower survival (nymph-to-adult:  $\chi^2_{(1)} = 102.3$ ; day 14:  $\chi^2_{(1)} = 106.8$ ,  $P < 0.001$  in both cases), a significant reduction in body size (tibia length [mm]:  $\chi^2_{(1)} = 7.51$ ,  $P < 0.01$ ) and in fecundity ( $t_6 = 3.7$ ,  $P < 0.05$ ).

#### 3.2. Within-generational and transgenerational effects of a food-restricted environment on $F_1$ aphids

The results of the present investigation show that the nutritional characteristics of the maternal environment influenced the life history traits of the offspring generation (Table 1). Nymph-to-adult survival and survival at 12, 16 and 20 days were significantly affected by maternal and current nutritional environment, but the interaction between these two factors was never found to be significant (Table 1, Fig. 2).  $OF_1$  aphids reared on plants had a higher survival than  $PF_1$  individuals reared on leaves. The survival of offspring whose mothers developed in an optimal environment increased compared to offspring whose mothers developed in a poor-resource one ( $OF_0-OF_1 > PF_0-OF_1$  and  $OF_0-PF_1 > PF_0-PF_1$ ), indicating the existence of plasticity effects and possible maternal ones (Fig. 2).

Similar to survival, the current nutritional environment of aphids always had a significant effect on their fecundity, while the maternal

environment influenced fecundity only during the intervals between days 9–12 and days 17–20. The interaction between the two factors was never found to be significant (Table 1). Except for the time interval between day 13 and day 16, the fecundity of the offspring is higher when their mothers have been developed on a broad bean plant (the Optimal scenario), regardless of the current environment. Aphids developed on a plant always had a higher fecundity compared with the fecundity of individuals developed on cut leaves (Fig. 3).

There were significant effects of the maternal and current environment on adult body size (Fig. 4), with measures of tibial length increasing in the optimal current environment (O) or when mothers developed in it. There was no significant interaction between the maternal and the current environment (Table 1).

There was a significant effect of the maternal environment on starvation tolerance measured at 24 h (Fig. 5). More interestingly, there was a significant interaction between the maternal environment and current environment, indicating the existence of transgenerational plasticity (Table 2). The starvation tolerance of  $OF_0-PF_1$  was higher than that of  $OF_0-OF_1$ , while the resistance of  $PF_0-PF_1$  was lower than that of  $PF_0-OF_1$ . It is interesting to note that the resistance to starvation of aphids whose mothers were reared in the resource-poor environment (P) increased significantly compared to those whose mothers were reared in the optimal environment (O). The group that performed better in terms of resistance to starvation after 24 h was  $PF_0-OF_1$ .

After 48 h without food, the survival of aphids decreased significantly (Fig. 5), but no significant effects of the maternal and current environment, or their interaction, were found.

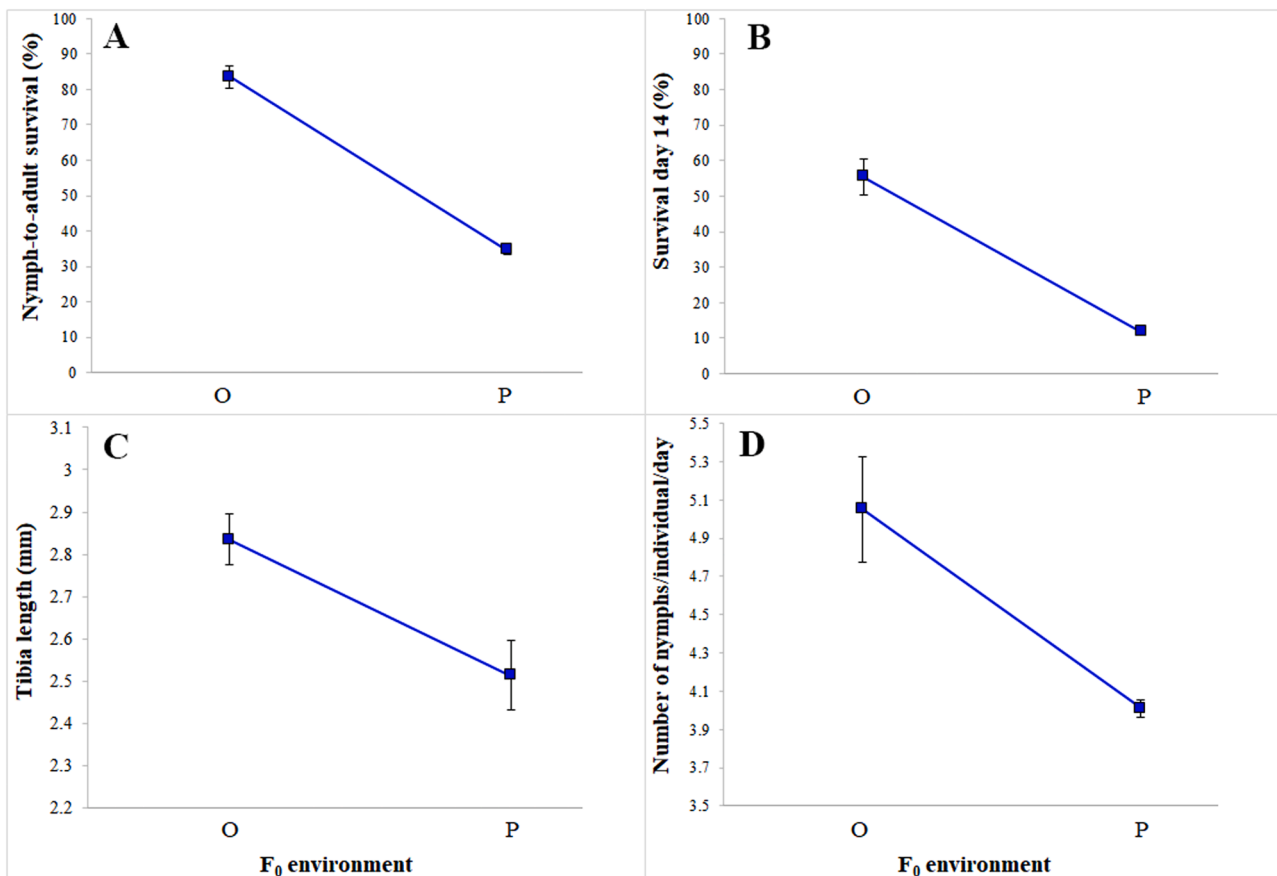


Fig. 1. Life history traits of *A. pisum* in the  $F_0$  generation as a function of optimal and food-restricted environments. Data are presented as mean  $\pm$  SE. (A) Nymph-to-adult survival; (B) survival at day 14; (C) body size measured as tibial length [mm]; (D) fecundity measured as the number of nymphs / individual / days. O, optimal resource environment (plant); P, poor-resource environment (leaves).

**Table 1**

Results of GLMs testing the effects of “maternal” and “current” environment on the F<sub>1</sub> aphid survival, fecundity, and adult body size. Survival was analyzed during the pre-adult period (interval between day 0 and day 8) and after 12, 16, and 20 days after deposition. Aphid fecundity was analyzed at three-time intervals: days 9–12, days 13–16, and days 17–20.

Survival										
Effect	d.f.	Nymph-to-adult		Day 12		Day 16		Day 20		
		$\chi^2$	P-value	$\chi^2$	P-value	$\chi^2$	P-value	$\chi^2$	P-value	
Maternal	1	20.4	6.2e-06	16.8	4.1e-05	17.1	1.6e-08	21.9	2.8e-06	
Current	1	47.8	4.8e-12	29.9	4.4e-08	32	3.6e-05	31.8	1.7e-08	

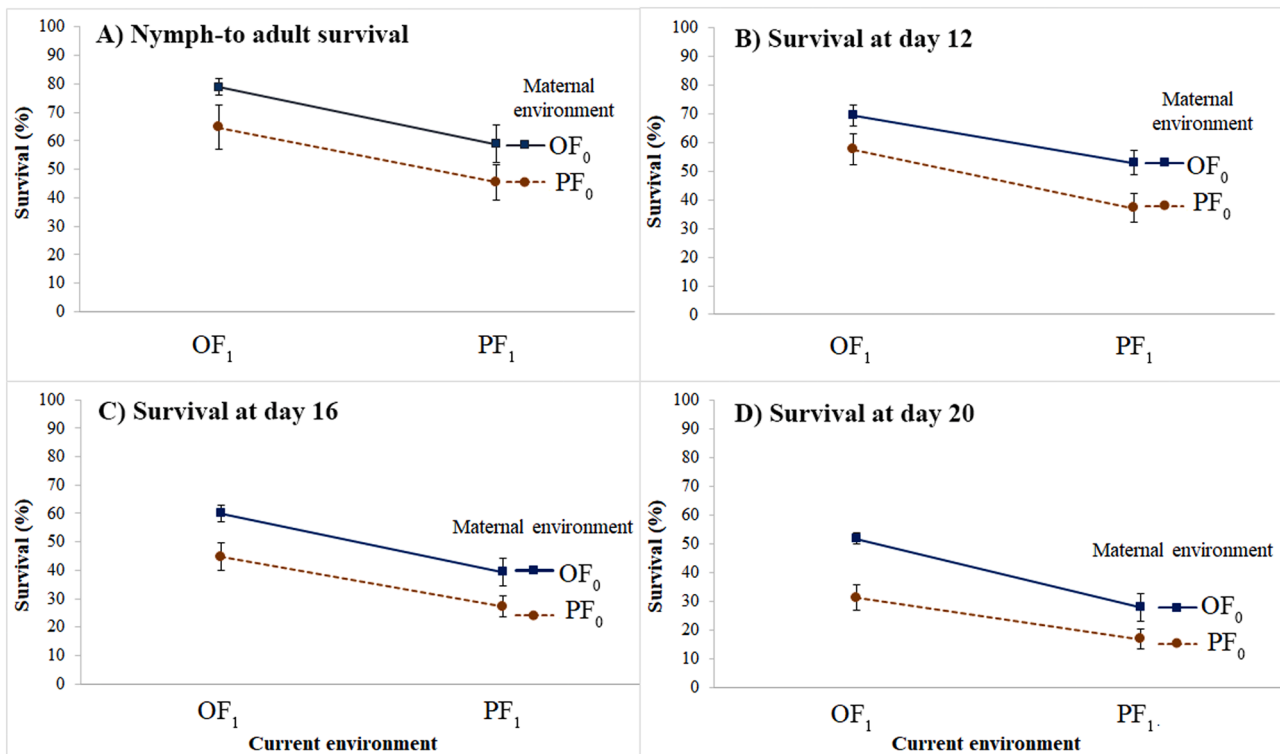
  

Fecundity										
Effect	d.f.	Days 9–12			Days 13–16			Days 17–20		
		Sum Square	F	P-value	Sum Square	F	P-value	Sum Square	F	P-value
Maternal	1	11.9	6.9	0.014	7.6	2.1	0.16	8.4	11.1	0.003
Current	1	16.4	9.6	0.0048	23.8	6.5	0.017	3.3	4.4	0.047
Residuals	25/25/21	42.9			91.5			15.8		

Body size				
Effect	d.f.	$\chi^2$	P-value	
Maternal	1	4.4	4.1e-05	
Current	1	7	0.0081	

d.f., degrees of freedom;  $\chi^2$ , chi-square tests; F, variance ratio.



**Fig. 2.** Mean percentage of survival ( $\pm$  standard errors) of *Acyrthosiphon pisum* in the F<sub>1</sub> generation as a function of optimal (O) and poor-resource (P) rearing environments. (A) Nymph-to adult survival; (B) survival at day 12; (C) survival at day 16; (D) survival at day 20. F<sub>0</sub> refers to the environment in which the mother developed. F<sub>1</sub> refers to the current environment in which the aphids developed.

**4. Discussion**

Phenotypic plasticity is very important for organisms to cope with adverse conditions experienced within a generation. In nature, however, the adverse conditions may persist for more than one generation. Organisms can influence the performance of their offspring through maternal effects, when the maternal environment determines the response, or through transgenerational plasticity, when the parental environment shapes the plasticity of the offspring. Transgenerational effects can be considered adaptive if the current and maternal

environments are similar and the transgenerational phenotypic changes induced by the parents can enhance the fitness of the offspring (Agrawal et al., 1999; Herman and Sultan, 2011).

The results of the present study suggest a variety of fitness costs associated with the development of aphids under resource scarcity and, consequently, a reduction in their life history traits (Dixon, 1987). Aphids reared in a poor-resource environment (in our case, a pair of cut leaves) always exhibited reduced body size, reduced survival, and reduced reproductive potential, consistent with a systemic effect probably due to a change in amino acid concentrations in the phloem. The

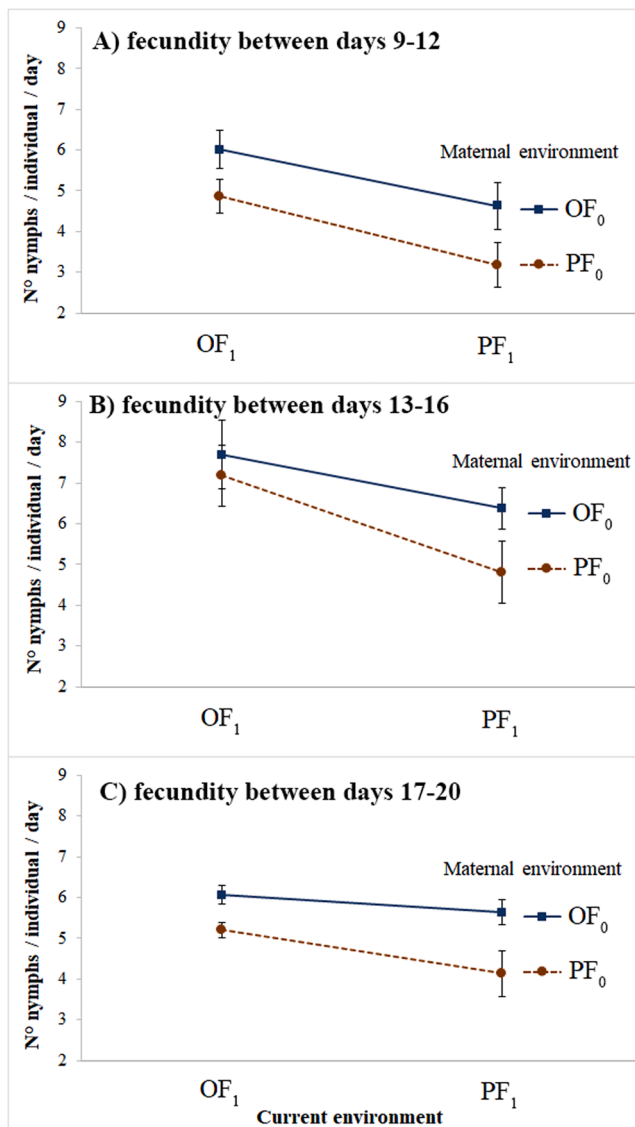


Fig. 3. Mean fecundity ( $\pm$  standard errors) measured as the total number of nymphs per adult per day of *Acyrthosiphon pisum* in the F<sub>1</sub> generation as a function of optimal (O) and poor-resource (P) rearing environments. (A) fecundity between days 9–12; (B) fecundity between days 13–16; (C) fecundity between days 17–20. F<sub>0</sub> refers to the environment in which the mother developed. F<sub>1</sub> refers to the current environment in which the aphids developed.

reduction in body size observed in our experiments is similar to that observed with a reduction in plant quality (Chiozza et al., 2010; Leckstein and Llewellyn, 1973). Adult body size is a valid measure of aphid performance since it is often positively correlated with fecundity and offspring size (Murdie, 1969; Ríos Martínez and Costamagna, 2018). However, we cannot assume that “bigger” is always “better”, as there are costs associated with larger body size that reduce overall fitness: prolonged developmental time, increased juvenile mortality and the probability of predation.

We also showed that a poor maternal environment always constrains the body size, survival, and fecundity of the next generation. This has been reported in previous studies (Hayden et al., 2021; Zehnder et al., 2007). However, trans-generational effects on organismal development are related to resource abundance, as parents reared in a resource-limited environment produce offspring of lower quality due to reduced nutrient supply (Baker et al., 2018; Herman and Sultan, 2016).

Repetitive stressful environments are expected to elicit an adaptive response, but the direct role of stress in shaping the genetic architecture

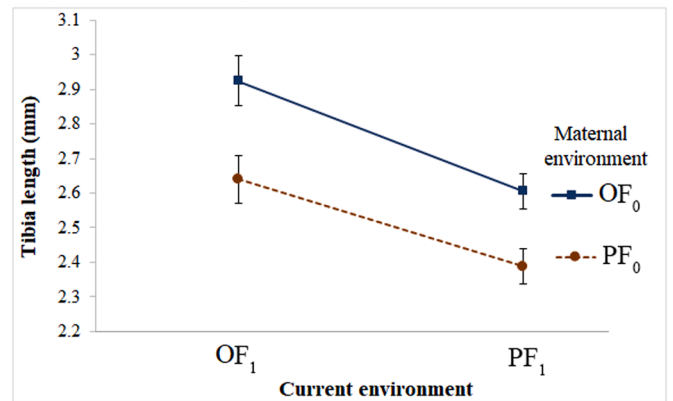


Fig. 4. Mean body size ( $\pm$  standard errors) measured as the length of the hind tibia of adult *Acyrthosiphon pisum* individuals in the generation F<sub>1</sub> as a function of optimal (O) and poor-resource (P) rearing environments. F<sub>0</sub> refers to the environment in which the mother developed. F<sub>1</sub> refers to the current environment in which the aphids developed.

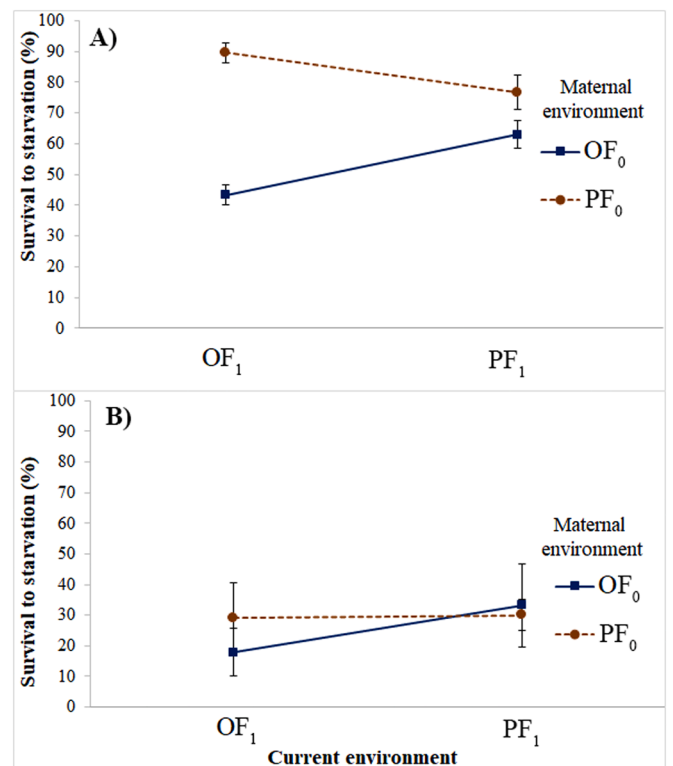


Fig. 5. Mean values of starvation tolerance ( $\pm$  standard errors) of *Acyrthosiphon pisum* in the F<sub>1</sub> generation as a function of optimal (O) and poor-resource (P) rearing environments. Starvation tolerance was estimated as the number of aphids alive relative to the initial number of aphids after 24 h of starvation (A) and after 48 h (B). F<sub>0</sub> refers to the environment in which the mother developed. F<sub>1</sub> refers to the current environment in which the aphids developed.

of life history traits is not obvious (Hoffmann and Parsons, 1997). If an aphid is developing with limited food resources, it is reasonable to assume that the quality of the host plant will deteriorate over time. The challenge for the aphids, as for other arthropods, is either to escape or to adapt to new conditions. Consistent with other studies (Berryman and Hawkins, 2006; Costamagna and Landis, 2011; Ríos Martínez and Costamagna, 2018), we observe a reduced population growth in response to resource limitation.

However, we observed that when an aphid is reared in a poor-

**Table 2**

Results of GLM testing the effects of “maternal” and “current” environments on starvation resistance after 24 h without food in the wet environment and in the dry environment.

Effect	d.f.	$\chi^2$	P-value
Maternal	1	13.6	0.00022
Current	1	0.05	0.82
Interaction	1	6.01	0.014

d.f., degrees of freedom;  $\chi^2$ , chi-square tests.

resource environment, it produces more starvation-tolerant offspring, regardless of its current environment. Similar results have been found in other model systems. For example, in two species of *Daphnia* (Anomopoda: Daphniidae), mothers reared with low food levels produced offspring, albeit few in number, that were able to survive long periods of starvation (Gliwicz and Guisande, 1992). It has also been shown that, in the guppy *Poecilia reticulata* Peters, parents reared under stressful conditions can produce offspring with increased tolerance to food scarcity, depending on the environment of the offspring (Bashey, 2006). In our experiment, the increased starvation tolerance was clearly evident in the offspring of mothers reared in the poor-resource scenario, which showed the greatest reductions in body size, survival, and fecundity. The TGP we observed for starvation tolerance under our experimental conditions could be interpreted as a form of plasticity resulting from an evolutionary bet-hedging strategy, with an intra- and inter-generational mix of fitness costs and gains. Fitness variation can generally be adaptively reduced by natural selection (Cohen, 1966), but in variable environments phenotypic plasticity can achieve the same results (Via and Lande, 1985). Theory predicts that when rapid environmental fluctuations (including resource availability) are highly unpredictable in natural environments, organisms may be forced to adopt a fitness strategy without knowing the consequences for their fitness. These “evolutionary bet-hedging” strategies (Slatkin, 1974) imply a reduction in average fitness, but also a positive reduction in fitness variance (Cohen, 1966). The increased starvation tolerance shown by the offspring of mothers reared in the poor-resource scenario could be interpreted as a kind of conservative “bet-hedging”: mothers produced fewer and lower-quality offspring, but with increased resilience when the nutritional environment deteriorated sharply, thus avoiding risky investments. This may be because the energy reserves of the offspring have been altered and redirected towards survival as a result of an adaptive change in the reproductive state of the mothers (Bell and Bohm, 1975; Brough and Dixon, 1990; Xu et al., 2012). Mothers, in turn, could have transmitted this information transgenerationally to their offspring, making them potentially more resistant in the absence of food, but with lower fitness, regardless of the current environment. However, because stressed viviparous aphids contain embryos that carry their own offspring (Kindmann and Dixon, 1989), we do not know where the boundary lies between plasticity and transgenerational effects.

The plastic response of most life history traits to resource limitation is often not considered adaptive, but merely the result of developmental constraints or physiological trade-offs. In this sense, the failure of an organism to buffer its growth against environmental stress may be the cause of the observed trait variation (Smith-Gill, 1983), and its adaptive value is difficult to determine. Despite the apparent fitness costs, our results suggest that adaptive TGP effects may be common in aphids and are very important for fully understanding the population dynamics of this insect pest. The effects of TGP observed in response to starvation may provide a method for aphids to rapidly induce a defensive strategy, posing new challenges for active integrated pest management, as TGP represents a potential resource that allows a population to rapidly adapt to natural and anthropogenic stressors.

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## CRedit authorship contribution statement

**Vincenzo Trotta:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Pierluigi Forlano:** Methodology, Validation, Writing – review & editing. **Vittoria Caccavo:** Methodology, Validation, Writing – review & editing. **Paolo Fanti:** Methodology, Validation, Writing – review & editing. **Donatella Battaglia:** Methodology, Investigation, Validation, Resources, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

All data presented in this article are available in a single Excel file (see Supplementary File 1).

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.cris.2024.100081](https://doi.org/10.1016/j.cris.2024.100081).

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