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Contrasting Phenotypic Variability of Life-History Traits of Two Feral Populations of *Macrolophus pygmaeus* (Hemiptera: Miridae) under Two Alternative Diets

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Abstract: *Tuta absoluta* is a major pest attacking tomato crops. This invasive species emerged in Europe (Spain) in 2006, and 3 years later it spread to Portugal. In 2009/2010, it was recorded for the first time in the Azores archipelago. *Macrolophus pygmaeus* is a predator widely used as biological control agent against the tomato leaf miner. This study contrasted the life-history traits and population growth parameters of two feral populations of *M. pygmaeus*, one from Portugal mainland and one from the Azores archipelago. The predators were tested on single prey diet, either of *Ephestia kuehniella* eggs, a factitious prey used for mass rearing of mirids, or *T. absoluta* eggs. We predicted that populations would express differences in its phenotypic characteristics, with the Azorean population displaying low performance due to likely low genetic diversity, as expected for insular populations. Our results revealed the inexistence of phenotypic differences in several life history traits, such as immature developmental time, female longevity, males' body weight and sex ratio. Contrary to our predictions, traits with direct impact on fitness, such as lifetime fertility (95.78 ± 14.23 vs. 61.38 ± 13.52 nymphs), explain better performances for the population of the Azores. Azorean *M. pygmaeus* females were larger, matured earlier and reproduced at a higher rate for longer periods, than mainland females. Therefore, population growth parameters show a positive advantage for the population of the Azores, fed on *T. absoluta* (time required for doubling the population Azores, Ek: 8.42 ± 0.50, Ta: 5.76 ± 0.31 and mainland, Ek: 10.88 ± 1.94, Ta: 12.07 ± 3.15). Biological performance of *M. pygmaeus* was similar when fed with *T. absoluta* or *E. kuehniella* that could be beneficial both to optimize mass production of the predator and biological control of the pest. Our results are discussed as well in a fundamental perspective, seeking if differences in biological performance can be explained by lower genetic diversity driven by geographic isolation.

Keywords: tomato crop; *Tuta absoluta*; *E. kuehniella*; phenotypic variability; life-history traits; population growth parameters

1. Introduction

Biological control of agricultural pests is a fundamental strategy for sustainable food production, as it provides a safe alternative to chemical control. However, classical approach of importing and introducing exotic natural enemies to control herbivorous pest populations poses some concerns in conservation biology [1,2]. One possible approach to control non-native pest populations is the use of native natural enemies, based on conservation biological control or augmentative releases [3].

The South American leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an alien species and a major pest of tomato crops in Europe, causing severe economic losses to growers [4]. The pest was firstly recorded outside of its native range in Eastern Spain in 2006, and it is now widely distributed in the Mediterranean Basin, including North Africa and Middle East [5,6]. In Portugal mainland, *T. absoluta* has been reported in protected tomato crops, since 2009 [7–9]. In the Azores archipelago (Portugal), the species was recorded in São Miguel Island in 2009/2010 [10]. Later on, in 2014/2015, populations were recorded in other islands, including Terceira, Faial, Pico and São Jorge Islands, infesting tomato crops in greenhouses and open fields [11]. More recently, it was observed in greenhouses of Graciosa Island (A.O. Soares, personal observations in 2020).

Macrolophus pygmaeus Rambur (Hemiptera: Miridae) is an omnivorous mirid, widely used in integrated pest management, especially against pest populations of vegetable crops, including *T. absoluta* in greenhouses and fields tomato crops. It has been successfully used in the regulation of other small arthropod pests, such as whiteflies, thrips, spider mites and aphids [12–22]. Due to its success, *M. pygmaeus* is among the most widely used biocontrol agents [3]. *Macrolophus pygmaeus* was reported to the Azores archipelago in 2012 [23]. However, the origin and date of colonization process are unknown. We can assume that Azorean populations were originated in Portugal mainland. Sanchez et al. [24] recognized three geographically based genetic populations, including one from the Iberian Peninsula and the Canary Islands. Sanchez et al. [24] found that Canary Islands populations present a lower allelic richness and expected heterozygosity, due to the bottleneck effect, and this might be a consequence of the loss of diversity in the process of colonization of the islands from the mainland.

Despite the extensive use of *M. pygmaeus* as biocontrol agent, many knowledge gaps persist, particularly concerning the genetic diversity of naturally occurring or mass-produced released insects [25], and how that diversity depict phenotypic variation in biological and ecological traits, such as host specificity, voracity, phytophagy, life-history, population growth parameters, and effectiveness as biological control agent. Previous studies about the genetic structure of naturally occurring or mass reared *M. pygmaeus* revealed differences among populations [24,25]. The context of the evolutionary history of *M. pygmaeus* may have contributed to the variation of the genetic structure in the Mediterranean area. Likely, isolation of *M. pygmaeus* during the last glaciations, when northern and center of Europe were covered either by ice fields or permafrost [26], might have had an important impact once geographically isolated populations evolved independently and may have become genetically differentiated [24]. Additionally, some results support the hypothesis that mass reared and "wild" populations of *M. pygmaeus* are genetically differentiated [25]. This is particularly important in understanding of how *M. pygmaeus* may perform in different agroecosystem contexts, and to support better designing biological control tactics by selecting the most effective biocontrol agents [25].

In this context, the present study aimed at contrasting life-history traits and population growth parameters, in two distinct feral populations of *M. pygmaeus*, one from Portugal mainland and one from Azores archipelago, fed on single prey diets of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, a factitious prey used for mass rearing of predatory mirids, in comparison with *T. absoluta* eggs. From an applied point of view, we intend to test the ability of the predator from both populations to complete development and reproduction, assessing its potential to be mass-reared and to be used as biocontrol agent of this tomato pest. In a more fundamental perspective and considering the expected low

genetic diversity of *M. pygmaeus* of insular population, such as that observed in Canary Islands, in comparison with Iberian Peninsula [24], we expected to find differences in the phenotypic characteristics of mainland and insular populations of *M. pygmaeus*.

2. Materials and Methods

2.1. Biological Material

Feral specimens of *M. pygmaeus* collected from two geographically distinct populations (Azorean and Portugal mainland) were brought to the laboratory. Mainland specimens were originated from a rearing established population, to which were added periodically collected specimens, mainly from the Western region of Portugal (Azueira, Mafra, 39°00′43.8″ N 9°16′49.1″ W; Silveira, Torres Vedras, 39°07′02.5″ N 9°21′54.1″ W; Tapada da Ajuda, Lisbon, 38°42′45.8″ N 9°11′02.8″ W), both in greenhouse and open field crops. The Azorean specimens were collected in S. Miguel Island, in five different locations, on tomato plants (37°47′48.1″ N 25°35′51.7″ W; 37°45′4.3″ N 25°34′27.2″ W; 37°48′49.59″ N 25°31′41.10″ W; 37°45′1.54″ N 25°41′28.70″ W; 37°45′1.54″ N 25°41′28.70″ W). Insular and mainland stock populations were kept in separate rooms inside rearing cages ($40 \times 40 \times 40$ cm^3) covered by mousseline fabric. One potted tomato plant (var. Nacera, Gebroeders Bakker Zaadteelt en Zaadhandel B.V., Netherlands) with approximately 3 months old was put in each rearing cage and *E. kuehniella* eggs were provided twice a week, as a source of animal protein. The diet was complemented with droplets of honey diluted in water (1:1), distributed over the leaflets. When necessary, the tomato plant was replaced by a new one. Rearing conditions were 25 ± 1 °C, $75 \pm 5\%$ relative humidity and light regime 16L:8D.

Ephestia kuehniella was mass-reared in a facility of the Biotechnology Centre of Azores, of the University of the Azores. The lepidopteran eggs for use in the experimentations were irradiated with UV light for 20 min to stop embryonic development. Then, the eggs were stored at 5 °C for use up to 1 month.

Tuta absoluta was reared at a small scale, according to the needs of the ongoing experimentations. Adults were collected in a greenhouse (37°45′19.28″ N 25°37′42.51″ W) and brought back to the laboratory to establish a trophic chain. Adults were kept in a rearing cage ($40 \times 40 \times 40$ cm^3) covered with mousseline fabric, with a tomato plant leaf in a water pot to collect lepidopteran eggs. Droplets of honey diluted in water (1:1) were put over the tomato plant leaflets for adult feeding. For the experiments, the tomato leaf containing the eggs was retrieved 24 h later and put over a dated paper sheet and kept at 5 °C to a maximum of 10 days. A preliminary test showed that after 10 days at 5 °C the viability of *T. absoluta* eggs was not affected. Additionally, at this temperature, the embryonic development was negligible after 10 days. When transferred to 25 °C these eggs took 4–5 days to hatch, practically the same time if kept always at 25 °C. This method allowed us to obtain a continuous supply of *T. absoluta* eggs at the required amounts for the experimentations. For trophic chain maintenance, the tomato leaf was kept up to 5 days in the adult rearing cages and then transferred to plastic boxes with a paper towel on the bottom and fresh tomato plant leaves. Fresh food for the larvae was provided as necessary until pupation. Newly emerged adults were collected and transferred to the adult rearing cage.

2.2. Life-History Traits

2.2.1. Immature Development

To determine the effect of different diets (*E. kuehniella* vs. *T. absoluta* eggs) and different populations (Azorean vs. Portugal mainland) on the developmental time of *M. pygmaeus*, approximately 50 *M. pygmaeus* adults from the stock population were introduced in a new rearing cage for 24 h, as described in Section 2.1, biological material. One tomato leaf inserted in a water pot was put inside the rearing cage with *E. kuehniella* eggs and a few droplets of honey diluted in water (1:1) were placed on the leaflets. Considering

the extremely low fecundities of *M. pygmaeus* when fed with *T. absoluta* [27,28], only *E. kuehniella* was provided to the mirid to obtain eggs.

To obtain enough nymphs (N = 30 for each diet, *E. kuehniella* or *T. absoluta* eggs) for the experiment, two rearing cages were set. These procedures were the same for the Azorean and mainland populations. From the 9th day on, tomato leaves were checked daily under stereomicroscope for the presence of newly hatched nymphs, to determine the embryonic development time. Each newly hatched nymph was transferred individually to a plastic box (3 cm Ø × 2 cm height) containing one tomato plant leaflet with *E. kuehniella* or *T. absoluta* eggs and one droplet of honey diluted in water (1:1). Nymphs were fed *ad libitum* (see additional details in Section 2.1). Every 2 days, the tomato plant leaflet was replaced. The presence of nymph exuviae was used as evidence of moulting and so the nymphal stage-specific and total immature development time were determined. At emergence, gender of each individual was determined based on the abdomen format, rounded for females and streamlined for males, and the individuals were weighed (10^{-4} mg Mettler AM50 analytical balance).

2.2.2. Longevity, Sex-Ratio and Reproductive Performance

To determine the reproductive performances of *M. pygmaeus* on the two diets tested, couples of each population were formed (N = 9) and kept isolated in Twin-Vented Mini Vivarium ($10 \times 8.8 \times 21.5$ cm^3) + Water pot boxes (Bugzarre), along with a section of a tomato plant leaf with approximately 10 cm long stem containing a single leaflet. Eggs of *E. kuehniella* or *T. absoluta* were placed *ad libitum* on the leaflet every day, according to the diet regime. *Tuta absoluta* eggs provided were obtained as described in Section 2.1, ensuring that only eggs were available to predator. To complement diet, a droplet of honey diluted in water (1:1) was put over the leaflet every 2 days. In case of uneven sex ratio obtained in the development time experiment, males from the stock population were used to make as many couples as possible. Every day, the tomato plant leaf was replaced by a new one and the old one was labelled and kept in water for the next 9 days. At the 9th day, the leaves were removed from water and transferred individually to plastic boxes (10 cm Ø × 3 cm height), for observation under the stereomicroscope (for the next 5 days) to check for the presence of newly hatched nymphs and determine the pre-oviposition time and the daily reproductive rate. As *M. pygmaeus* females lay their eggs inside the tomato plant stem, they are very difficult to observe; therefore, reproductive performance of the predator was assessed based on the number of nymphs obtained. The females were followed until their death and males were substituted whenever they died.

2.3. Population Growth Parameters

To calculate the intrinsic rate of increase (r_m), the Euler's equation was solved iteratively [29–31] according to Carey's method [32]:

$$1 = \sum e^{-r_m x} l_x m_x$$

where x is time (in days), l_x is the age-specific survivorship and m_x is the age-specific mean number of female offspring. The number of females produced by female, that is the net reproductive rate (R_0), was calculated as [33,34]:

$$R_0 = \sum l_x m_x$$

The time required for a population to increase in size by a factor of R_0-fold (the mean generation time, τ) was calculated according to Southwood & Henderson [35]:

$$\tau = \frac{Ln R_0}{r_m}$$

The finite rate of increase (λ) and the doubling time (DT), were calculated as previous described [30,36–38]:

$$\lambda = e^{r_m}$$
$$DT = \frac{Ln2}{r_m}$$

2.4. Statistical Analysis

We used generalized linear models (GZLM) to assess the effect of population origin and food resource (independent variables) on the developmental time, sex-ratio, females and males body weight, pre-oviposition time, lifetime fertility, length of the oviposition period and female longevity (dependent variables) of *M. pygmaeus*. Factors (Mainland vs. Azorean populations and *E. kuehniella* vs. *T. absoluta* eggs), and interactions were analyzed using the Wald Chi-Square test for a confidence level of 95%. Pairwise multi-comparisons were performed, and *p* values corrected using Bonferroni test. Due to the lack of normality of the data on the developmental time of eggs, a Mann–Whitney test was used to compare the populations (Mainland vs. Azorean populations) feed with *E. kuehniella* eggs. Normal distributions of data were assessed by the Kolmogorov–Smirnov test. Accordingly, for dependent variables with normal distribution, analyses considered the normal error distribution and the identity link function. For dependent variables without normal distribution, we used Poisson error distribution and the log link function.

To compare population growth parameters, pseudo-values (P_j) of the parameters were calculated using jackknife analysis according to the formula [39]:

$$P_j = n \times E_{all} - (n-1) \times E_{-j}; \ j = 1, \ldots, n$$

where *n* is the number of replicates, E_{-j} is the estimate omitting female *j* and E_{all} is the estimate comprising all females. With *n* pseudo-values of the parameters, it is then possible to calculate a mean value (jackknife estimate of the statistic) and the respective standard errors (SE). Life table parameters for the two populations (island and mainland) on different diets (*E. kuehniella* or *T. absoluta* eggs) were compared with a GZLM, to assess the effect of population origin and food resource (independent variables) on population growth parameters (dependent variables) of *M. pygmaeus*. Factors (Mainland vs. Azorean populations and *E. kuehniella* vs. *T. absoluta* eggs), and interactions were analyzed using the Wald Chi-Square test for a confidence level of 95%. Pairwise multi-comparisons were performed, and *p* values corrected using Bonferroni test.

For survival analysis, the Online Application for Survival Analysis (OASIS 2) [40] was used. The survival curves intersected each other, therefore Cox Proportional Hazards and Log-Rank tests are inappropriate for data analysis because the hazards are not proportional [41–44]. Instead, a weighted Log-Rank test, the Fleming-Harrington test, was used to compare survival in early and late stages of life applying the Bonferroni correction.

All means in text and tables were calculated with untransformed data, followed by standard errors. All the statistical analyses were performed on the IBM SPSS 27.

3. Results

3.1. Life-History Traits

3.1.1. Immature Development

We found a significant difference in the embryonic development time of *M. pygmaeus* from Mainland and Azorean populations fed with *E. kuehniella* eggs (Mann–Whitney test, $U = 705.0$, $N = 60$, $p < 0.001$) (Table 1). For total developmental time of immature stages, we did not find a significant effect of the population origin and food resource (Wald Chi-Square ≈ 0, $df = 1$, $p = 0.993$). No differences were found between immature developmental time of Mainland (17.47 ± 0.11) and Azorean populations (18.35 ± 0.09) (Wald Chi-Square = 0.739, $df = 1$, $p = 0.390$), as well as between the offered preys, i.e., *E. kuehniella* eggs (17.74 ± 0.12) versus *T. absoluta* eggs (18.09 ± 0.12) (Wald Chi-Square = 0.118, $df = 1$, $p = 0.731$) (Table 1). Survival rates of the immature stages of *M. pygmaeus* were high for the Azorean population (Table 2).

Table 1. Developmental time (days $\pm$ SE) of the embryo and immature stages of *Macrolophus pygmaeus* from the Azorean and Portugal mainland populations fed on single diets of *Ephestia kuehniella* or *Tuta absoluta* eggs (25 $\pm$ 1 °C; 75 $\pm$ 5% relative humidity and light regime 16L:8D).

	Azores		Portugal Mainland	
	E. kuehniella (N)	*T. absoluta* (N)	*E. kuehniella* (N)	*T. absoluta*(N)
Egg	11.37 $\pm$ 0.11 (30) a		12.00 $\pm$ 0.00 (30) b	
Immature	18.18 $\pm$ 0.10 (17) a	18.53 $\pm$ 0.15 (17) a	17.29 $\pm$ 0.17 (17) a	17.65 $\pm$ 0.12 (17) a

Different letters mean significant differences (Mann–Whitney test or GZLM, $p < 0.05$).

Table 2. Survival rate of the immature stages of *Macrolophus pygmaeus* from the Azorean population and Portugal mainland fed on single diets of *Ephestia kuehniella* or *Tuta absoluta* eggs (25 $\pm$ 1 °C; 75 $\pm$ 5% relative humidity and light regime 16L:8D).

Azores		Portugal Mainland	
E. kuehniella (N)	*T. absoluta* (N)	*E. kuehniella* (N)	*T. absoluta*(N)
0.85 (17)	0.96 (17)	0.73 (17)	0.69 (17)

3.1.2. Longevity, Sex-Ratio and Reproductive Performance

The results about longevity, sex-ratio and reproductive performance of *M. pygmaeus* from the Azorean and Portugal mainland populations fed on single diets of *T. absoluta* eggs or *E. kuehniella* eggs are presented in Table 3.

Table 3. Adults' life-history traits of *Macrolophus pygmaeus* of the Azorean and Portugal mainland populations fed on single diets of *Ephestia kuehniella* or *Tuta absoluta* eggs (25 $\pm$ 1 °C; 75 $\pm$ 5% relative humidity and light regime 16L:8D). The information on the two additional columns (significance) stands for biological traits in which no significant effect between factors we found (origin of population and food resource).

	Azores		Portugal Mainland		Significance	
Biological Traits	*E. kuehniella* (N)	*T. absoluta* (N)	*E. kuehniella* (N)	*T. absoluta* (N)	**Region**	**Prey**
Sex-ratio (Females:Males)	0.65:0.35	0.65:0.35	0.42:0.58	0.42:0.58	n.s.	n.s.
Female body weight (mg)	1.09 $\pm$ 0.03 (17)	1.10 $\pm$ 0.03 (17)	1.04 $\pm$ 0.05 (8)	0.97 $\pm$ 0.04 (8)	Az. > P.m.	n.s.
Male body weight (mg)	0.65 $\pm$ 0.04 (9)	0.68 $\pm$ 0.03 (8)	0.67 $\pm$ 0.02 (11)	0.61 $\pm$ 0.03 (8)	n.s.	n.s.
Pre-oviposition time (days)	5.88 $\pm$ 1.62 (8) a	4.11 $\pm$ 0.42 (9) a	7.17 $\pm$ 2.26 (6) a	14.33 $\pm$ 4.94 (6) b		
Lifetime fertility (n. of nymphs)	77.89 $\pm$ 19.14 (9)	113.67 $\pm$ 20.36 (9)	44.89 $\pm$ 18.51 (9)	35.00 $\pm$ 14.46 (9)	Az. > P.m.	n.s.
Oviposition period (days)	26.51 $\pm$ 3.16 (8)	22.00 $\pm$ 2.89 (9)	20.17 $\pm$ 4.66 (9)	16.00 $\pm$ 3.07 (9)	n.s.	n.s.
Female longevity (days)	48.44 $\pm$ 5.34 (9)	38.33 $\pm$ 5.80 (9)	54.78 $\pm$ 8.69 (9)	50.56 $\pm$ 7.97 (9)	n.s.	n.s.

Az., P.m. and n.s. stands for Azorean population, Portugal mainland population and no significant, respectively. Different letters mean significant differences ($\chi2$ or GZLM, $p < 0.05$).

No significant female-biased sex-ratio were obtained when comparing population origin (Chi-Square = 2.166, p = 0.141) as well as food resource (Chi-Square = 0.259, p = 0.611).

Comparing female body weight of *M. pygmaeus*, we did not find a significant effect between factors (origin of population and food resource: Wald Chi-Square = 1.143, df = 1, p = 0.285). Body weight of females from mainland (1.00 $\pm$ 0.02 mg) was significantly lower than of the Azorean females (1.01 $\pm$ 0.02 mg) (Wald Chi-Square = 6.797, df = 1, p = 0.009). In terms of food resources, females body weight did not significant differ when fed with *E. kuehniella* (1.07 $\pm$ 0.03 mg) or with *T. absoluta* eggs (1.05 $\pm$ 0.02 mg) (Wald Chi-Square = 0.730, df = 1, p = 0.393).

In relation to male's body weight, we did not find a significant effect between factors (origin of population and food resource: Wald Chi- Square = 1.962, df = 1, p = 0.161). Body weight of males from mainland (0.63 $\pm$ 0.02 mg) did not differ from that of the

Azorean males (0.66 ± 0.02 mg) (Wald Chi-Square = 0.605, *df* = 1, *p* = 0.437). In terms of food resources, males body weight did not significant differ when fed with *E. kuehniella* (0.66 ± 0.02 mg) or with *T. absoluta* eggs (0.64 ± 0.02 mg) (Wald Chi-Square = 0.238, *df* = 1, *p* = 0.625).

Concerning pre-oviposition time, we found a significant effect between factors (origin of population and food resource: Wald Chi-Square = 13.257, *df* = 1, *p* < 0.001). Pre-oviposition duration in mainland females fed on *T. absoluta* was significantly higher than the remaining results.

In terms of lifetime fertility, we did not find a significant effect between factors (origin of population and food resource: Wald Chi-Square = 1.760, *df* = 1, *p* = 0.185). Lifetime fertility of mainland females (39.94 ± 11.46 nymphs) was significantly lower than Azorean females (95.78 ± 14.23 nymphs) (Wald Chi-Square = 10.524, *df* = 1, *p* = 0.001). In terms of food resources, fertility did not significantly differ when females were fed with *E. kuehniella* (61.39 ± 13.52 nymphs) or *T. absoluta* eggs (74.33 ± 15.42 nymphs) (Wald Chi-Square = 0.566, *df* = 1, *p* = 0.452).

Concerning the oviposition period of *M. pygmaeus* females, we did not find a significant effect between factors (origin of population and food resource: Wald Chi-Square $\approx$ 0, *df* = 1, *p* = 0.995). Oviposition period of mainland females (18.09 ± 2.73 days) did significantly differ from the Azorean females (23.94 ± 2.13 days) (Wald Chi-Square = 3.478, *df* = 1, *p* $\leq$ 0.062). Oviposition period also did not significantly differ when females fed on *E. kuehniella* (23.57 ± 5.74 days) or *T. absoluta* eggs (19.60 ± 2.20 days) (Wald Chi-Square = 1.672, df = 1, *p* = 0.196).

For female longevity of *M. pygmaeus*, we did not find a significant effect between factors (origin of population and food resource: Wald Chi-Square = 0.194, *df* = 1, *p* = 0.660). Longevity of mainland females (52.67 ± 1.71 days) did not significantly differ from the Azorean females (43.39 ± 4.02 days) (Wald Chi-Square = 1.925, *df* = 1, *p* = 0.165). Longevity also did significantly differ when females were fed on *E. kuehniella* (51.61 ± 5.01 days) or *T. absoluta* eggs (44.44 ± 5.01 days) (Wald Chi-Square = 1.148, *df* = 1, *p* = 0.289).

3.2. Population Growth Parameters

The results of population growth parameters of *M. pygmaeus* are presented in Table 4. The information on the two additional columns (significance) stands for population growth parameter in which no significant effect between factors was found (origin of population and food resource).

Table 4. Population growth parameters of *Macrolophus pygmaeus* from the Azorean and Portugal mainland populations fed on single diets *Tuta absoluta* or *Ephestia kuehniella* eggs (25 ± 1 °C; $75 \pm 5\%$ relative humidity and light regime 16L:8D).

	Azores		Portugal Mainland		Significance	
	E. kuehniella	*T. absoluta*	*K. kuehniella*	*T. absoluta*	**Region**	**Prey**
r_m	0.082 ± 0.005 a	0.114 ± 0.004 b	0.062 ± 0.009 ac	0.054 ± 0.010 c		
λ	1.085 ± 0.005 a	1.121 ± 0.004 b	1.064 ± 0.009 ac	1.056 ± 0.010 c		
R_0	41.16 ± 10.12 a	74.86 ± 12.78 b	15.25 ± 5.13 a	12.09 ± 4.19 a		
τ	45.72 ± 1.09 ab	36.69 ± 0.64 a	45.47 ± 2.30 ab	47.37 ± 4.45 b		
DT	8.42 ± 0.50	5.76 ± 0.31	10.88 ± 1.94	12.07 ± 3.15	Az. < P.m.	n.s.

Az., P.m. and n.s. stands for Azorean population, Portugal mainland population and no significant, respectively. Different letters mean significant differences (GZLM, *p* < 0.05).

Concerning the intrinsic rate of increase (r_m: Wald Chi-Square = 8.189, *df* = 1, *p* = 0.004) and the finite rate of increase (λ = Wald Chi-Square = 8.652, *df* = 1, *p* = 0.003), we found a significant interaction between factors (origin of population and food resource). We found that the intrinsic rate of increase and the finite rate of increase, were significantly higher for Azorean females fed on *T. absoluta* and lower for mainland females, especially fed on *T. absoluta*.

For net reproductive rate (R_0), expressing the number of females produced by female, we found a significant effect between factors (origin of population and food resource: Wald Chi- Square = 4.938, *df* = 1, *p* = 0.026). Again, net reproductive rate was significantly higher for Azorean females fed on *T. absoluta* and no difference were found between the remaining populations and food resources.

For mean generation time (τ), there was a significant effect between factors (origin of population and food resource: Wald Chi-Square = 5.041, *df* = 1, *p* = 0.025). Mean generation time are relatively similar but tendentially lower for Azorean females fed on *T. absoluta* and higher for mainland females fed on *T. absoluta*.

For doubling time (DT), the time required for doubling the population, we did not find a significant effect between factors (origin of population and food resource: Wald Chi-Square = 1.192, *df* = 1, *p* = 0.275). Time required for doubling the population of mainland (11.4 ± 1.2 days) was significantly higher than for Azorean population (7.1 ± 1.2 days) (Wald Chi-Square = 6.165, *df* = 1, *p* = 0.013). In terms of food resources, doubling time did not significantly differ from females fed with *E. kuehniella* (9.6 ± 1.2 days) and with *T. absoluta* eggs (8.9 ± 1.2 days) (Wald Chi- Square = 0.173, *df* = 1, *p* = 0.677).

Fertility curves showed that reproductive investment of *M. pygmaeus* females were distributed within a similar time window (30–70 days) for island and mainland populations on both diets, but the island population produced much more eggs. The mainland population presented lower survival early in life but mortality during adult stage showed a steady decreasing pattern (Figure 1). Prey species and population origin did not affect significantly survival either on early (Azorean *E. kuehniella* vs. Azorean *T. absoluta*: Chi-Square = 0.040, *p* = 1; Azorean *E. kuehniella* vs. Mainland *E. kuehniella*: Chi-Square = 1.080, *p* = 0.895; Azorean *E. kuehniella* vs. Mainland *T. absoluta*: Chi-Square = 2.970, *p* = 0.254; Azorean *T. absoluta* vs. Mainland *E. kuehniella*: Chi-Square = 1.140, *p* = 0.856; Azorean *T. absoluta* vs. Mainland *T. absoluta*: Chi-Square = 3.170, *p* = 0.225; Mainland *E. kuehniella* vs. Mainland *T. absoluta*: Chi-Square = 2.780, *p* = 0.287) or late stages in life (Azorean *E. kuehniella* vs. Azorean *T. absoluta*: Chi-Square = 0.000, *p* = 1.000; Azorean *E. kuehniella* vs. Mainland *E. kuehniella*: Chi-Square = 4.100, *p* = 0.129; Azorean *E. kuehniella* vs. Mainland *T. absoluta*: Chi-Square = 0.740, *p* = 1.000; Azorean *T. absoluta* vs. Mainland *E. kuehniella*: Chi-Square = 4.040, *p* = 0.133; Azorean *T. absoluta* vs. Mainland *T. absoluta*: Chi-Square = 0.700, *p* = 1; Mainland *E. kuehniella* vs. Mainland *T. absoluta*: Chi-Square = 0, *p* = 1).
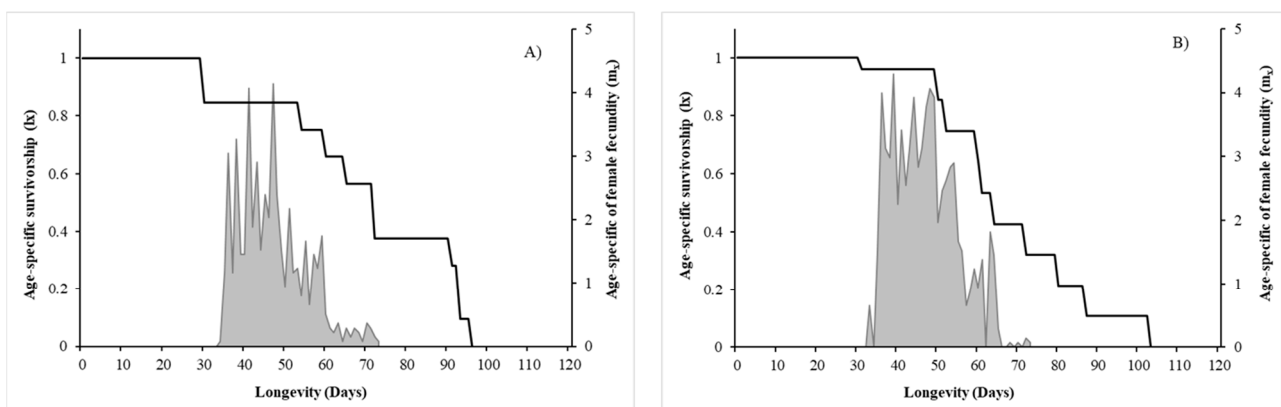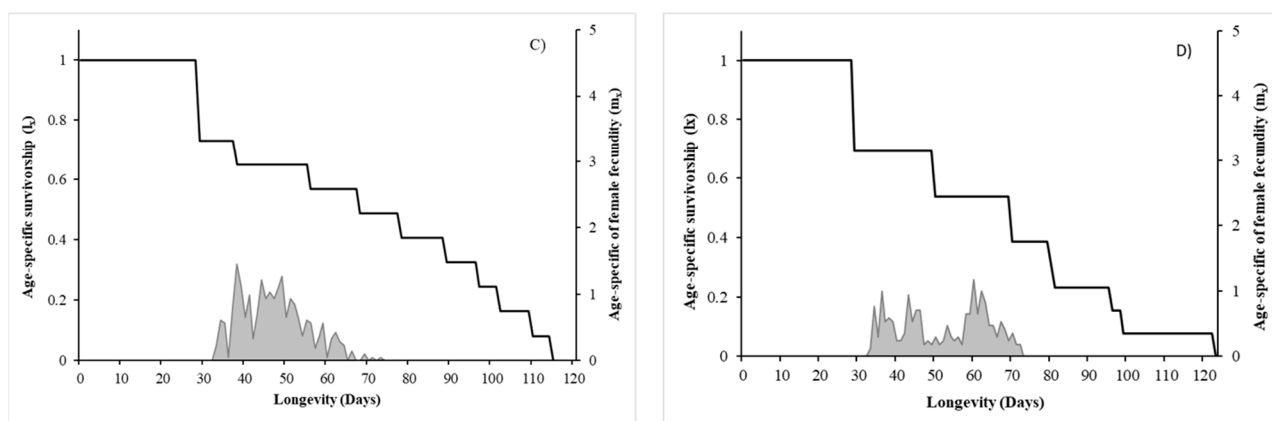

Figure 1. *Cont.*

Figure 1. Age-specific survivorship (l_x) and age-specific of female fecundity (m_x) curves of *Macrolophus pygmaeus* from the Azores fed on *E. kuehniella* (**A**) or *T. absoluta* (**B**), and from mainland fed on *Ephestia kuehniella* (**C**) or fed on *Tuta absoluta* (**D**). (25 ± 1 °C; 75 ± 5% relative humidity and light regime 16L:8D).

4. Discussion

We aimed at contrasting life-history traits and population growth parameters of *M. pygmaeus* from two different populations, one from Portugal mainland and the other from the Azores, either fed with *E. kuehniella* or *T. absoluta* eggs. To our knowledge, this is the first study on the life-history of feral populations of *M. pygmaeus*. We found that both preys were able to ensure the completion of the immature development of the predator, in about 18 days (between 17.29 ± 0.17 and 18.53 ± 0.15 days), with a high survival rate, varying from 69% to 96%. The registered developmental times were higher than the reported by Mollà et al. [27], both for *E. kuehniella* and *T. absoluta* diets and for both mainland and Azorean populations of *M. pygmaeus*. Females reached a similar body weight (approximately 1 mg), despite significantly higher for females of the Azores. Both preys allowed reproduction, but with a variable degree of fitness (differing from 35.00 ± 14.46 to 113.67 ± 20.66 nymphs produced), with an advantage for the Azorean population, independently of the prey consumed. Females' longevity was slightly longer when feeding on *E. kuehniella*, results that differ from those of Mollà et al. [27]. Although the progeny (number of nymphs/female) was more or less similar to that of these authors in case of the mainland population, Azorean population presented a higher lifetime fertility (number of nymphs produced, i.e., 113.67 and 77.89, feeding on *T. absoluta* and *E. kuehniella*, respectively in our study, in comparison with 44.5 and 49.2 nymphs/female, respectively in Mollà et al. [27]), and reproductive investment is distributed within a similar time window (30–70 days). These results corroborate that both preys are suitable food sources for *M. pygmaeus* and suggest it is an adequate candidate to be mass reared and used as biological control agent against *T. absoluta*. Indeed, previous studies demonstrated that *M. pygmaeus* consume *T. absoluta* eggs either under laboratory [20] and greenhouse [45,46] conditions. In short, biological performances of the predator were similar when the predator was fed with *T. absoluta* and *E. kuehniella*, which may have positive implication for mass production and biological control programs of this tomato pest. Nevertheless, this result is in contradiction with the reported by other researchers, which observed that this lepidopteran is a poor food resource for ovipositing females of *M. pygmaeus* [27,28]. For that reason, further studies are needed with other feral populations to confirm those results.

Translating our data of biological traits into the population growth parameters, we found a slightly advantage for the Azorean population of *M. pygmaeus* when fed with *T. absoluta* and a marginal disadvantage for mainland population. The intrinsic rate of increase (r_m) was higher, except for the mainland population fed with *T. absoluta* eggs. The time required for doubling the population (*DT*) was lower in the case of the Azorean population fed on *T. absoluta* and higher for mainland populations. Life table parameters

of *M. pygmaeus* are strongly dependent on temperature and food resources [47]. For instance, under experiments performed at 25 °C, *M. pygmaeus* fed on *Solanum nigrum* L. (Solanaceae) leaves infested with the aphid prey *Aphis fabae solanella* Theobald (Homoptera Aphididae), showed a lower performance (r_m = 0.0392, λ = 1.0399, R_0 = 5.76, τ = 46.04 and DT = 17.68), than the populations tested in our study, independently of the prey. The predator performed even worst when fed on *Dittrichia viscosa* L. (W. Greuter) (Asteraceae) leaves infested with the aphid *Capitophorus inulae* (Passerini) [48]. As *M. pygmaeus* is a zoophytophagous predator, the availability of prey, simultaneously with a host plant, is fundamental to ensure the development and reproduction of the predator [47]. Even in the absence of prey, eggplant is more suitable than tomato plant [49].

We predicted that the studied populations would express differences in its phenotypic characteristics with the Azorean populations displaying low performance due to likely low genetic diversity, as expected for insular populations, e.g., [24]. Indeed, for arthropods, there are growing evidence about the effect of insular conditions to inbreeding depression, loss of genetic diversity and mutation accumulation, and this contributes to increase extinction risk [50]. For some of the life-history traits, our results revealed the inexistence of phenotypic differences. This holds true for embryonic and immature developmental time, female longevity, males' body weight and sex ratios. Contrary to our predictions, traits with direct impact on fitness, such as females body weight, pre-oviposition time, oviposition period and lifetime fertility translated in better biological performances in the Azorean population. Azorean *M. pygmaeus* females were larger, matured earlier and reproduced at a higher rate for longer periods than mainland females. Pulling the individual data on phenotypic traits into the models of population growth parameters, we found a significant positive advantage for populations of the Azores. Indeed, the values of net reproductive rate, intrinsic rate of increase, finite rate of increase were significantly higher for Azorean females and consequently, mean generation time and doubling time were significantly lower. Thus, it is expected that Azorean populations of *M. pygmaeus* will achieve higher population densities more rapidly than that of mainland.

Both Mollá et al. [27] and Sylla et al. [28] studies have used mass-reared mirids acquired from mass rearing facilities. It is known that mass rearing insects for long periods may lead to decreased biological performance and adaptation to the food source used. In our study, we used feral populations reared in laboratory conditions and the number of generations was not enough to develop this adaptation, even because refreshments were made periodically. Furthermore, the mainland and Azorean populations were collected in areas without known previous release of *M. pygmaeus*. In mainland, collections occurred in Tapada da Ajuda, a green area inside the metropolitan area of Lisbon, and in Azueira, a farm surrounded by pear orchards, vineyards and forestry. Streito et al. [25], however, found that some of the supposedly feral populations might not be so and *M. pygmaeus* has been commercialized in Europe since 1994 [3]. Therefore, we should consider the possibility of the feral mainland population being affected by commercial populations released in the area or neighboring areas, as there is a large area of protected crops in the Western region ($\approx$860 ha [51]). By the way, this can be an explanation for the higher similarity between the mainland population and the results of Mollà et al. [27]. The release of conspecific populations may have different potential impacts in natural populations, including the loss of genetic variation, breakdown of adaptations, changes to genetic composition within populations, and breakdown of population structure [52]. For example, Sethuraman et al. [53] found that multilocus genotypes of the commercially distributed populations of the predatory lady beetle *Hippodamia convergens* (Coleoptera: Coccinellidae) from California were admixed into Eastern populations.

To understand if the lower biological performance of the mainland population is related with an unlikely lower genetic diversity, a study on the population genetic structure is required, sampling a much larger area together with historic information about biological control practices in the collection sites and their surroundings. Anyway, it is not always possible to relate biological or behavioural differences from the analysis of some genes [54].

Considering the recent arrival of *T. absoluta* to the Azores archipelago, the observed fitness of a feral population of *M. pygmaeus* on a new prey abundantly available in its habitat may have direct impact on *T. absoluta* control and on its diet breadth. More studies on other preys would contribute to clarify this question. It would be also important to address what will be the impact of releasing commercially available strains on the Azorean population of *M. pygmaeus*.

Conflicts of Interest: The authors declare no conflict of interest.

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