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Interference competition with an invasive species as potential driver of rapid extinction in an island-endemic lizard

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ABSTRACT

Competition between native and alien species is often described as a main driver of biodiversity loss. Nevertheless, there is limited evidence of animal declines and extinctions actually determined by competition. The Aeolian lizard, *Podarcis raffonei*, is critically endangered because it suffered dramatic declines and extinctions throughout its range. Competition and hybridization with invasive Italian lizards, *Podarcis siculus*, have been proposed as a driver of the shrinkage of Aeolian lizards, still the mechanisms underlying their decline remain poorly resolved. We used observations of behavioral encounters, combined with morphological data and robust species identification based on genomics, to test whether agonistic interactions with the invasive Italian lizard can explain the competitive exclusion and rapid decline of the native Aeolian lizard while accounting for hybridization. Invasive lizards were larger, with larger heads, and showed higher bite tendency against neutral items. In agonistic encounters between males, Aeolian lizards received more attacks and escaped more frequently than invasive males. The performance of Aeolian males was particularly poor in interspecific encounters. Genomic data verified that tested individuals were all pure *P. raffonei* or *P. siculus*, with a single hybrid individual detected. The strong competitive advantage of invasive males can allow them monopolizing territories, potentially hampering the reproduction of both native males and females, thus resulting in a mechanism of sterilizing interference. Reproductive interference competition mediated by spatial exclusion might be an unappreciated process determining rapid decline in endemic species. Safeguarding areas devoid of invasive species should be the priority strategy to avoid the extinction of the Aeolian lizards.

1. Introduction

Invasive alien species (IAS) are one of the greatest causes of recent biodiversity loss and have contributed to about one-third of

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recent animal extinctions (Blackburn et al., 2019). IAS can determine the decline and extinction of native species through multiple mechanisms, such as direct consumption (e.g. predation), parasitism, habitat modifications, hybridization, spread of diseases and interspecific competition (Diamond, 1989; Pyšek et al., 2020; Stewart, 1991; Strayer et al., 2006). Competition is a frequent process, and can drive native species declines, especially when invasives show superior competitive performance (e.g. faster growth, larger size, better dispersal, foraging ability, stress tolerance or population growth rate). Nevertheless, it is often assumed that competition alone can only rarely determine extinction (Pyšek et al., 2017; Pyšek et al., 2020). This occurs because the relative competitive ability of species can vary across environments and, under certain conditions (e.g. in specific habitat "refugia"), native species are expected to show better performance than the invasive ones (Case et al., 1994; Cole et al., 2005; Grabowski et al., 2009).

However, there are situations where the potential effects of interspecific competition might be stronger, such as when closely related species are involved, and when native species are restricted to small areas, such as islands (Browett et al., 2023; Robert et al., 2019). In this case, only a small range of conditions / resources are available to the native species (Browett et al., 2023), limiting the possibility to find habitat refugia. Insular endemics are among the organisms suffering the strongest effects by IAS, and their narrow range limits both population size and heterogeneity of environmental conditions (Bellard et al., 2017). Nevertheless, there is very limited information on whether the actual levels of competitions between native and invasive species migth, alone, determine a fitness loss that is compatible with rapid extirpation of native animals.

The capability to secure and exploit important resources (e.g. food, shelters, territories for the access to mates) is a key determinant of the outcome of competitive interactions and is particularly relevant when native species enter in contact with related IAS (Case et al., 1994; Cole et al., 2005). The access to these resources is often regulated by agonistic interactions between contenders, where the prevailing individuals can access high-quality resources or exploit safer habitat patches, while relegating competitors to suboptimal or risky patches (Alcock, 2013; Langkilde et al., 2005; Martin et al., 2017). Asymmetry in agonistic performance between invasive and native species is often alleged as a major determinant of the outcome of interspecific interactions occurring during biological invasions, potentially resulting in competitive exclusion and consequent native displacement or decline (Rowles and O'Dowd, 2007; Weis and Sol, 2016). For instance, stronger and more aggressive invasive crayfish can exclude other crayfish species from shelter use, increasing their exposure to predators and favouring their decline (Chucholl et al., 2008; Usio et al., 2001). Nevertheless, very limited data are available to estimate whether agonistic interactions can potentially determine range-wide decline and extinction of endemic species.

In this study, we used assessments of competitive performance to evaluate whether agonistic interactions between male lizards can potentially explain the extinction of insular-endemic species. As case study, we focused on the dramatic decline observed in one of the most endangered vertebrates of Europe, the Aeolian wall lizard (*Podarcis raffonei*). The Aeolian wall lizard is an island-endemic species that currently survives on three tiny islets scattered across the Aeolian archipelago plus a small peninsula of the Vulcano island and has one of the smallest ranges of all vertebrates in Europe (less than 2 ha) (Ficetola et al., 2021). Such a limited distribution is probably relictual of a wider range across the Aeolian archipelago (Capula, 1993; Capula, 1994; Capula and Lo Cascio, 2011). On Vulcano island, the species has suffered a >99 % range decline in the last 40 years at the advantage of the Italian wall lizard, *P. siculus*, that was introduced in historical times and is now widespread across the whole island except in a tiny peninsula (Capo Grosso), where *P. raffonei* holds its last stronghold (Capula et al., 2002; D'Amico et al., 2018; Ficetola et al., 2021; Lo Cascio, 2010; Sherpa et al., 2024; Fig. 1). Several hypotheses have been proposed to explain the dramatic decline of the Aeolian lizard, including habitat modifications, hybridization with the invasive Italian wall lizard, and / or differences in competitive performance (Capula and Lo Cascio, 2011; Capula et al., 2002; Ficetola et al., 2021; Gippoliti et al., 2017). In many lizard species, males perform agonistic competitive interactions to establish hierarchies and secure the control of territories with key resources such as food, shelters, thermoregulation sites and females (Donihue et al., 2016; Sacchi et al., 2009; Zambre et al., 2020). The outcome of male–male interactions is a stronger driver of variation in male reproductive success than female choice (Olsson and Madsen, 1998). As a consequence, interference competition between males can potentially drive processes of competitive exclusion and range displacement (Downes and Bauwens, 2002; Senior et al., 2021; While et al., 2015). This is a plausible scenario for wall lizards inhabiting the Aeolian archipelago as the invasive lizard is generally larger than the Aeolian lizard, shows remarkable phenotypic plasticity and high ability to adapt to island environments (Herrel et al., 2008; Sabolić et al. 2024; Sherpa et al., 2023). Interference competition could therefore explain the displacement of Aeolian lizards by invasive wall lizards (Corti et al., 2009), but experimental evidence supporting this hypothesis is limited.

In this study we assessed whether the difference in performance between invasive and Aeolian lizards during interspecific interactions supports a role of direct interference on the decline of Aeolian lizards. First, we tested whether invasive lizards possess morphological and behavioural traits favouring the outcome of interspecific interactions, such as head size and bite propensity. Second, we used behavioural assays to test whether the invasive lizard shows superior performance in agonistic interactions between males. Genome-wide single-nucleotide polymorphism (SNPs) allowed to ascertain the taxonomic identification of syntopic native and invasive lizards, and the occurrence of hybrids between them. Assessing whether interference competition is a likely driver of the decline of the Aeolian lizard has major implications for the conservation of this endangered species. If this hypothesis is verified, then management actions are needed to preserve strongholds in uninvaded areas. Alternatively, conservation attention should shift to different management scenarios.

2. Methods

2.1. Study species, study area and sampling

The critically endangered Aeolian wall lizard, *Podarcis raffonei* (hereafter Aeolian lizard), is endemic to the Aeolian Archipelago (Southern Italy). Its area of occupancy is estimated to be less than 2 ha, subdivided in three tiny offshore rocks and a small peninsula on

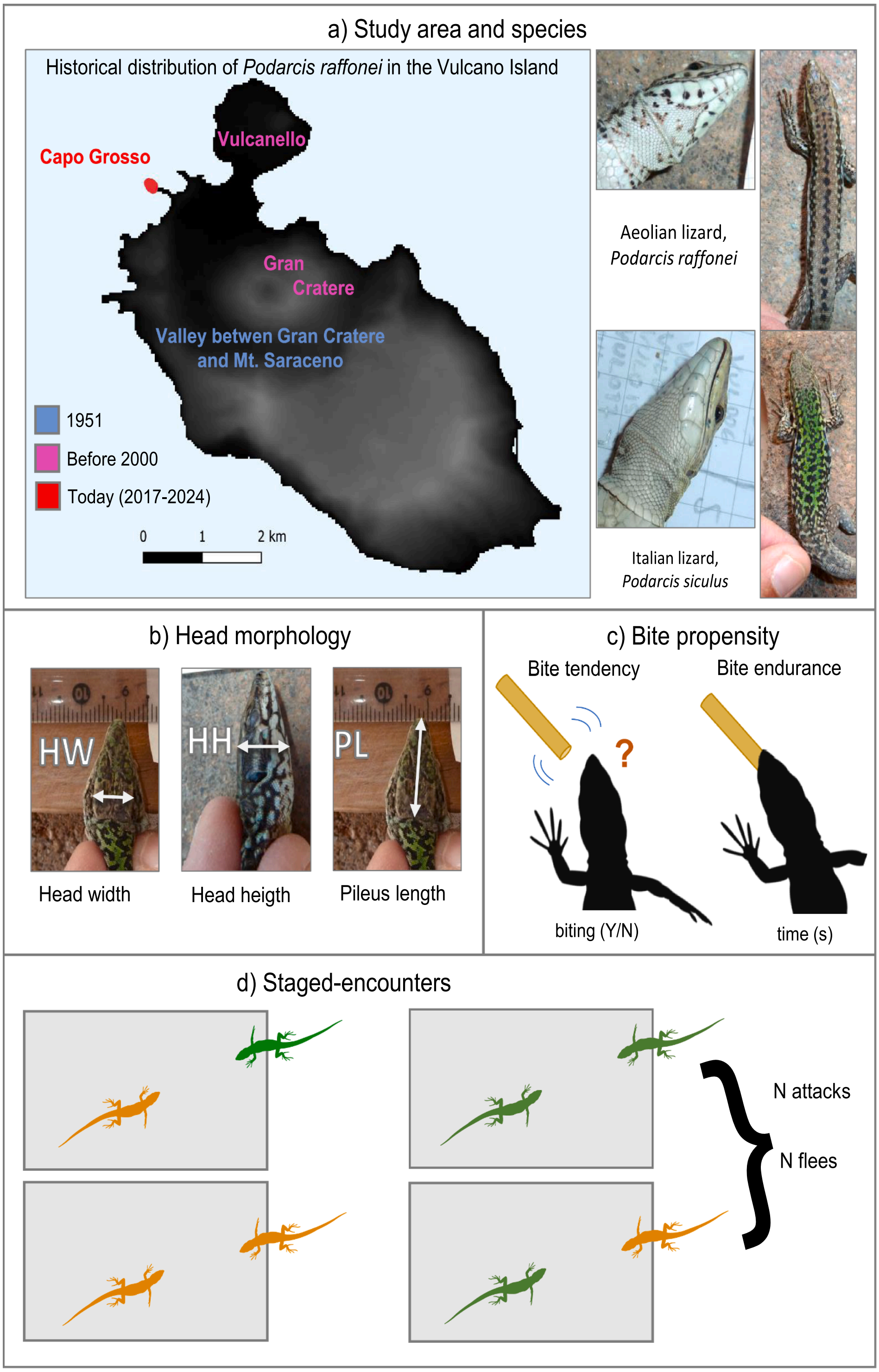

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Fig. 1. Study area, species and experimental setting. a): In the Vulcano Island, the endemic Aeolian lizard *Podarcis raffonei* suffered a dramatic decline in the decades. Blue: localities where the species was detected in 1951 (Mertens, 1955) but not confirmed subsequently; violet: localities where the species was detected in surveys performed before 2000, but not confirmed more recently (Capula, 1992; Capula, 1993; Capula et al., 2002; Lo Cascio, 2010; Capula and Lo Cascio, 2011; D'Amico et al., 2018; Ficetola et al., 2021); red: the last locality of Vulcano with recent records of *P. raffonei* (Lo Cascio, 2010; D'Amico et al., 2018; Ficetola et al., 2021). The interaction with the invasive Italian lizard, *P. siculus*, has been proposed as a main driver of the decline. We genetically identified 39 male Aeolian lizards and 25 invasive Italian lizards, and measured their b) head morphology and c) bite tendency. Furthermore, d) we assessed the outcome of interspecific and intraspecific agonistic interactions. In d), *P. raffonei* is depicted in orange, *P. siculus* is green. Individuals of the two species are tested both as residents or intruders in established territories.

the Vulcano island (Ficetola et al., 2021; Fig. 1; Gippoliti et al., 2017). This is generally interpreted as a relictual distribution, with the species assumed to be more widespread across the Aeolian Archipelago in the past (Capula, 1993). However, actual data on the decline of *P. raffonei* are only available for the Vulcano Island, where a dramatic decline has been observed since its discovery (>99 % decline in extent of occurrence, Fig. 1; Capula, 1993; Capula et al., 2002; D'Amico et al., 2018; Ficetola et al., 2021; Mertens, 1955). Within the Vulcano island, *P. raffonei* is currently restricted to the small Capo Grosso Peninsula (total area: ~0.7 ha) where less than 1 000 adults are estimated to survive (Ficetola et al., 2021; B. Gambioli and L. Vignoli unpublished data), whereas in two other locations (Vulcanello and Gran Cratere; Fig. 1), the species was last observed around 2000 (Capula et al., 2002; D'Amico et al., 2018; Lo Cascio, 2010). Conversely, the Italian wall lizard, *Podarcis siculus* (hereafter Italian lizard), is widespread in mainland Italy, Sicily and in many small islands, including the Aeolian Archipelago where it occurs in all the main islands and several islets (Bonardi et al., 2022; Lo Cascio and Corti, 2006). The Italian lizard has been introduced historically in Vulcano (Sherpa et al., 2024) and is currently widespread through this island (D'Amico et al., 2018). The two species are strongly differentiated, with fossil-calibrated phylogenetic trees suggesting a Miocene divergence (12–18 million years ago; Salvi et al., 2021; Yang et al., 2021).

In May 2017, we captured 66 adult male *Podarcis* lizards from the northern portion of the Vulcano Island on the Capo Grosso (38.418°N, 14.944°E) and Vulcanello (38.427°N, 14.958°E) peninsulas (Fig. 1). Vulcano is the only island where *P. raffonei* and *P. siculus* currently coexist, and thus where the mechanisms of interaction can be assessed (Bonardi et al., 2022; Capula et al., 2002). Lizards were collected by noosing and identified in the field based on the head shape and colour pattern (Capula and Lo Cascio, 2011). After capture, each animal was individually housed in an opaque plastic terrarium of 75 ×50 x 50 cm located outdoor in a fully fenced, shaded garden in Vulcanello, <1500 m from the sites of collection. The bottom of each terrarium was covered by local sand, and a small shelter (roughly 20 ×20 cm) built with two rocks and a tile, and a water reservoir were provided. The position of terraria was rotated so that each of them was exposed to the direct sunlight for ~2 h each day, during early morning or afternoon. Housing condition (opaque terraria) prevented males from observing each other before and after behavioural experiments, thus avoiding potential biases arising from prior experience. Lizards were fed in early morning and late afternoon with mealworm (*Tenebrio molitor*) larvae; experiments started at least 2 h after morning feeding. The average air temperature during the study period was 18.2°C.

2.2. *Experimental setting*

Before running the experiments, lizards were weighted (accuracy: 0.01 g) and measured with a calliper (accuracy: 0.1 mm). We recorded snout-vent length (SVL) and three parameters representing head size: maximum head width (HW); maximum head height (HH) and pileus length (PL) following standard guidelines (Kaliontzopoulou et al., 2012; Kaliontzopoulou et al., 2007) (Fig. 1). All these parameters show a very strong correlation with bite force and fighting success and are under strong sexual selection due to male-male competition (Kaliontzopoulou et al., 2012; López and Martín, 2001).

We used staged behavioural encounters to test differences in performance during aggressive interactions between males. For all the individuals, behavioural trials started 3 days after the capture date, to allow them establishing a territorial behaviour in their terrarium (Scali et al., 2021). Behavioural encounters were performed by introducing a lizard (intruder) into the terrarium where a second lizard was present since at least 3 days (resident) (Sacchi et al., 2009). Before each encounter, lizards were marked with a water-resistant colour to ensure individual recognition. The intruder was taken from his home terrarium and placed in an opaque plastic tube (length x diameter: 20 ×5 cm) for acclimation. The tube was placed next to the wall of the terrarium of the resident lizard, with a mobile septum separating the acclimation tube from the terrarium. After one minute, the septum was removed, and the intruder was free to enter the resident's terrarium. We used a Microsoft LifeCam HD 3000 to record the behaviour of lizards for 15 min after the first behavioural interaction between them. If no behavioural interaction occurred within 10 min from the entrance, the trial was interrupted and repeated the following day. The identity of test individuals was randomly selected so that each individual was subjected to a maximum of 6 trials, up to 3 as resident and up to 3 as intruder, always in different days and with different opponents, to avoid the effect of previous experience (López and Martín, 2001). Preliminary tests showed that the number of staged encounters experienced before the given trial has no effects on the outcome of trial itself, thus this parameter was not included into analyses. After 15 min the encounter was interrupted, the intruder was collected and placed in its home terrarium (López and Martín, 2001). To avoid injuries on lizards, we planned stopping interactions involving persistent attacks, but this was not necessary, as interactions either consisted of short chases and / or displays, or consisted of short bites but ended with the escape of one individual under the shelter. No individual suffered injuries or showed stress during or after the trials; all the individuals maintained an active behaviour and their body mass at the end of trials.

Five days after the end of behavioural trials, we recorded two measures of bite performance: bite tendency and bite endurance. In *Podarcis* lizards, bite performance is directly linked to aggressiveness and propensity to engage agonistic contests (Donihue et al., 2016). Bite tendency and endurance were measured for a subset of 42 lizards; each individual was tested in four trials during the same day, within the same timeframe used for behavioural encounters (9:00–13:00, 15:00–18:00). To measure bite tendency, the lizard was collected from its terrarium and a small (diameter: 5 mm) wood stick was moved six times left-to-right and back in front of its head, and we recorded whether the lizard bit the stick. If the lizard bit the stick, bite endurance was recorded as the length of the bite (in seconds). If endurance was >150 s, endurance test was ended and the trial received the maximum score (i.e. 150). Animals were left resting in their own terrarium for >30 min between consecutive replicates.

2.3. Genetic identification of lizards

Podarcis raffonei and *P. siculus* are morphologically difficult to identify (Capula, 1993) and, although dorsal and ventral colouration pattern are quite distinctive, some overlap occurs (Capula and Lo Cascio, 2011; Ficetola et al., 2021). Moreover, hybrids between the two species have been detected in the Vulcano island and show a mixture of morphologic and chromatic characteristics of both species, being extremely difficult to identify (Capula, 1993; Capula and Lo Cascio, 2011; Ficetola et al., 2021). Therefore, we relied upon genetic data for the taxonomic identification of tested individuals using a double-digest restriction-site associated DNA sequencing (ddRAD-seq) performed on an Illumina Hi-Seq 2500 plaftorm (Fasteris, Switzerland). The protocol and downstream analyses are explained in detail in Paris et al. (2024). In brief, DNA was extracted from tail tips, collected from each individual after behavioural trials, and digested using the restriction enzymes *SbfI* and *MspI* (New England Biolabs Inc.). In a preliminary analysis we also included pure *P. raffonei* individuals from Scoglio Faraglione and pure *P. siculus* from Milazzo as species reference. After ligation of standard Illumina adapters, libraries were obtained by PCR amplification with Taq-Phusion HF (New England Biolabs) at selected size of 250–500 bp with 12 cycles, and sequenced paired-end (2 ×125 bp). We used Stacks v2.60 (Rochette et al., 2019) for data cleaning and demultiplexing (*process_radtags*) and to obtain single-nucleotide polymorphisms (SNPs; Catchen et al., 2013) using either the *de_novo* pipeline or the *ref_map* pipeline by mapping reads to the reference genome of *Podarcis muralis* (PodMur_1.0; Andrade et al., 2019). We also explored loci assembly and parameter optimization separately for *P. raffonei* and *P. siculus* (Cerca et al., 2021; Paris et al., 2017). We obtained 84,083 loci and 189,763 variant sites loci with the *denovo* pipeline, with parameters -M=2 and -n=2, that were processed using a 'whitelist' procedure (see Paris et al., 2024) and filtered in vcftools v0.1. 17 (Danecek et al., 2011), keeping only biallelic sites at a genotype quality of 30. We obtained a 'linkage-pruned' dataset, with a single SNP for each RAD locus (–write-single-SNP), comprised of 2623 variant sites, and a 'full haplotype' dataset, with all SNPs for each RAD locus, of 17,254 variant sites. A principal component analysis (PCA) was performed using the linkage-pruned dataset in Plink 1.9 (Purcell et al., 2007) based on Pairwise-FST was calculated in Hierfstat v0.5.11 (Goudet, 2005). We then used the full haplotype information in Admixture 1.3.0 (Alexander et al., 2009) to estimate individual ancestries by varying the coancestry clusters (*K*) from 2 to 7 with a tenfold cross-validation (CV=10). Individuals were identified as *P. raffonei* or *P. siculus* based on *i*) their placement in the genetic space defined by the PCs relative to pure *P. raffonei* and *P. siculus* individuals, and *ii*) if they had >90 % assignment probability to the groups including pure *P. raffonei* and *P. siculus* individuals in the Admixture analysis. Individuals were classified as hybrids if placed in the genetic space in between the two species in the PCA and if they had ≤90 % assignment probability to either group in the Admixture analysis.

2.4. Behavioural characterization of agonistic interactions

Tendency to attack or to escape rivals are clear markers of agonistic asymmetry in male lizard interactions, being strongly associated to dominance or subordination, respectively (Sacchi et al., 2009). Accordingly, we measured agonistic performance by recording the number of attacks and of escapes by the two opposing lizards. As attacks, we considered rapid movements towards the opponent individual terminating with a bite attempt, a fight between the lizards, or a chasing of the opponent; escapes were recorded when the focal lizard performed a swift withdrawal movement at the sight the opponent or in response to behavioural interactions with it. The video analysis of agonistic encounters was performed using BORIS, an event-logging software for behavioural observations, that allows designing customised ethograms to simultaneously track the behaviour and interactions of multiple subjects (Friard and Gamba, 2016). Using BORIS we analysed behavioural displays (i.e. number of attacks and number of escapes) of both resident and intruder lizard. The lizards involved in each encounter were alternatively considered as focal lizard (i.e. the lizard that is the subject of observation, either as resident or as intruder; Bosholn and Anciães, 2017) or as opponent (with the opposite role). Video analysis was performed blindly by two operators unaware of the typology of trial they were analysing; preliminary analyses confirmed that the two operators recorded nearly identical numbers of attacks and escapes.

2.5. Statistical analyses

Only one individual was identified as hybrid between *P. raffonei* and *P. siculus* (see the Result section). Therefore, we focused analyses on the comparisons between the pure native (*P. raffonei*) and invasive (*P. siculus*) species.

First, we used linear models to compare the basic biometric parameters (SVL, weight HW, HH and PL) between *P. raffonei* and *P. siculus* males. We focused on absolute values of head size (as opposite to size-corrected), as they are the best predictors of biting performance (Kaliontzopoulou et al., 2012).

Second, we used generalized linear models (GLMM; individual identity included as random factor) to test differences between species in bite frequency and bite endurance. For bite tendency, the dependent variable was the tendency of lizards biting the wood stick during the trial (Y/N) and we used a binomial error. For bite endurance, the dependent was bite duration (in seconds, log-transformed), only considering trials with a successful bite attempt.

Finally, we used GLMMs to assess differences between species in the number of attacks and escapes during the agonistic interactions between males. The number of attacks / escapes by each individual in each trial was the dependent variable; we used a negative binomial error to take into account overdispersion. Fixed factors included species identity of the focal individual, species identity of the opponent, the interaction between them, and role (resident vs. intruder). As random factors, we included identity of the individual, identity of the opponent and ID of the behavioural trial. In lizards, the largest individuals tend to have better performance in male-male interactions (López and Martín, 2001), and the Italian lizard tends to be larger than the Aeolian lizard (Ficetola et al., 2021). We did not include the difference in body size between the target individual and the opponent (López and Martín, 2001) as covariate, because the very strong correlation between SVL and species identity (point-biserial correlation: $r = 0.61$, $P < 0.0001$) would cause collinearity issues, affecting the performance of the model (Dormann et al., 2013). Still, we repeated models including SVL differences and excluding species identity (Table S1). To confirm that the two species did not differ in their acclimation time, we also repeated analyses by including the interaction between species identity of test individuals, and number of days of acclimation. This model yielded identical results to the one without acclimation time; the interaction did not affect the number of attacks ($z = -0.628$, $P = 0.530$) nor the number of escapes ($z = 0.014$, $P = 0.989$), suggesting that the two species did not show differences in the time required to consider terraria their territory. In the Results section, we report results for the most parsimonious model, not considering acclimation time. We used the marginal R^2 (R^2_M) as an estimate of the amount of variation solely explained by the fixed effects of mixed models; for models with negative binomial error we estimated R^2_M using a trigamma approximation (Nakagawa et al., 2017).

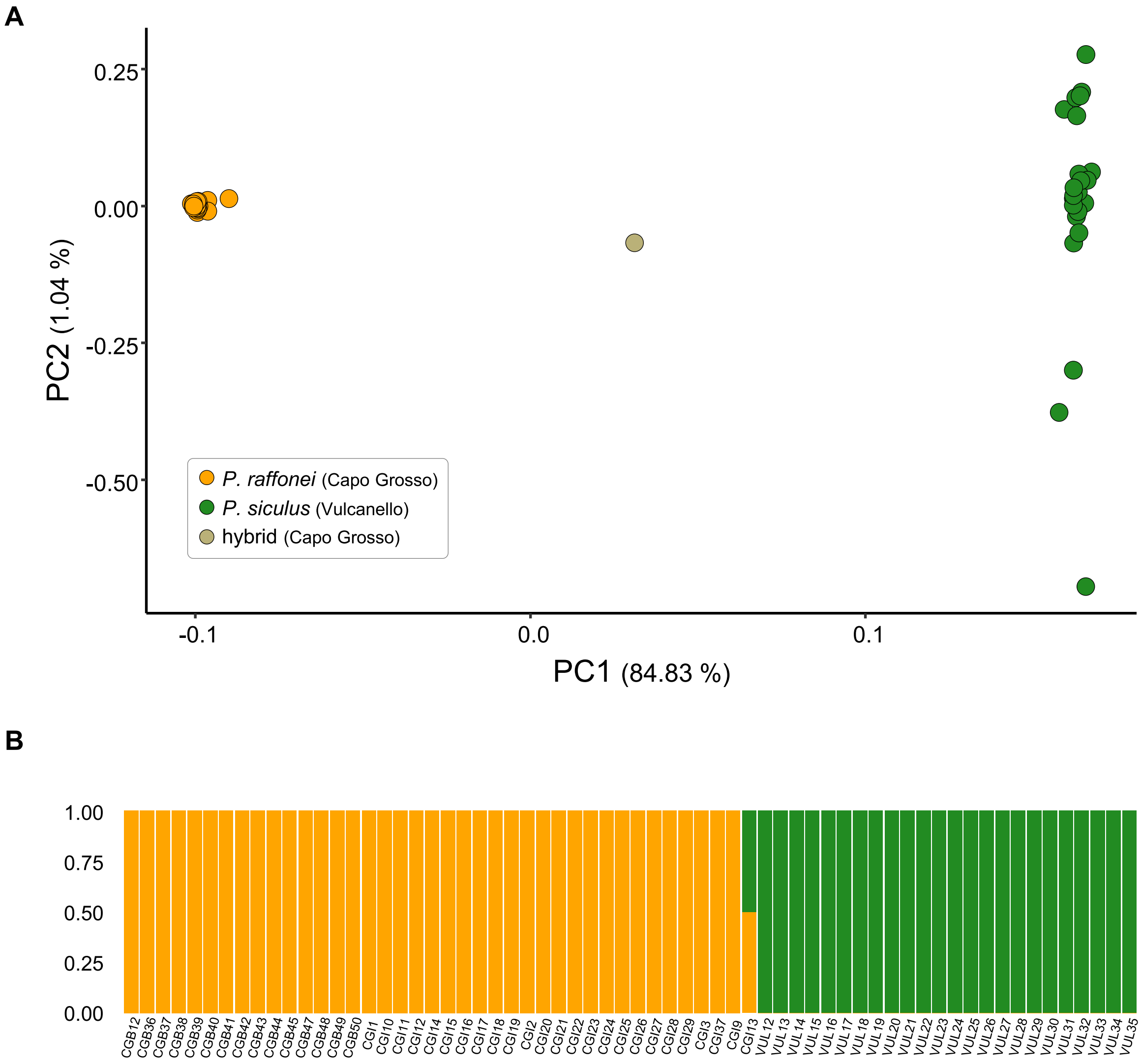

Fig. 2. Genetic assignment of test individuals. A) Principal Components Analysis (PCA). Green circles represent *P. siculus* lizards; orange circles represent *P. raffonei*; the pale green circle indicate the single detected hybrid. The plot also reports the amount of variance explained by PC1 and PC2. B) Individual ancestries estimated using Admixture (K=2).

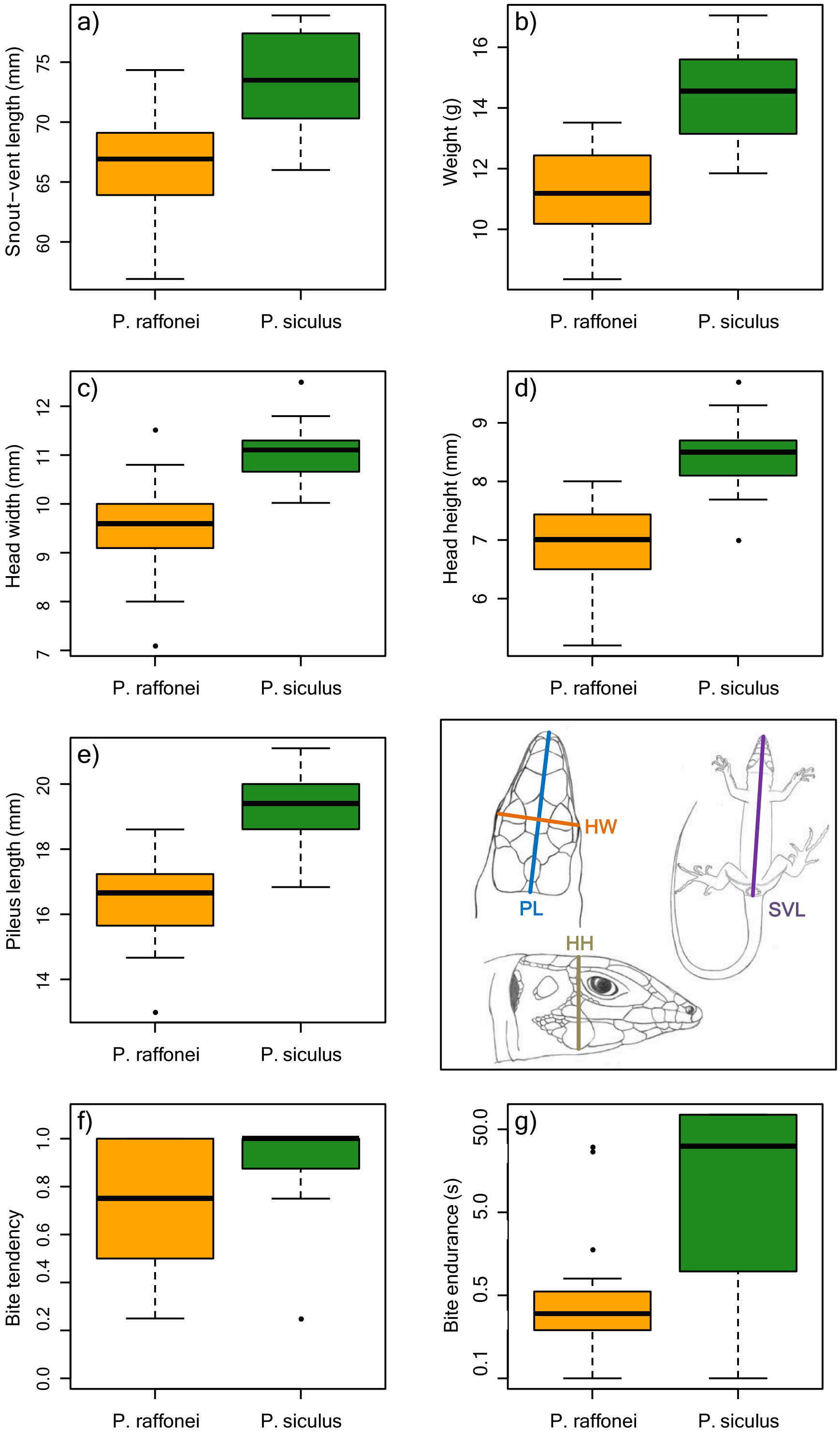

Fig. 3. Box-plot showing the variation of morphological features (a: snout-vent length, b: weight; c: head width, d: head height, d: pileus length) and of bite propensity (f: bite tendency; g: bite endurance) in trials against a neutral item. For all comparisons, $P < 0.05$.

3. Results

3.1. Genetic assignment

We successfully genotyped 65 adult males. One individual failed the RAD experiment (insufficient number of reads) and could not be genetically assessed, and was therefore excluded from the analyses. The two species showed a strong genetic differentiation in the PCA, were well separated in the genetic space (Fig. 2a) and represented two clearly defined coancestry clusters in the admixture plot (Fig. 2b). Based on PCA and Admixture analyses of genome-wide SNPs data we identified 39 pure Aeolian lizards, 25 pure Italian lizards, and just one hybrid. The hybrid individual was placed in the genetic space between the two species in the PCA analyses and showed approximately 50 % assignment probability to either species, therefore it likely represents a first-generation hybrid (F1) between the two species (Paris et al., 2024).

3.2. Morphological differences and bite tendency

Aeolian lizards were significantly smaller than Italian lizards for all the morphometric parameters (SVL: $t_{62} = 6.3$, $P < 0.001$; weight: $t_{62} = 6.2$, $P < 0.001$; HW: $t_{62} = 7.8$, $P < 0.001$; HH: $t_{62} = 9.4$, $P < 0.001$; PL: $t_{60} = 9.9$, $P < 0.001$; Fig. 3a-e).

Both species responded to the stimulus provided by the neutral item (wood stick) and bit it, but Aeolian lizards showed a lower bite tendency (average frequency ± SE: 0.73 ± 0.04 for Aeolian lizards; 0.90 ± 0.04 for Italian lizards; GLMM: $\chi^2_1 = 5.0$, $P = 0.025$; Fig. 3f). Furthermore, bites of Aeolian lizards lasted much shorter (average length: 5.6 ± 1.9 sec. for Aeolian lizards; 77 ± 8.3 sec. for Italian lizards; GLMM: $t_{35.4} = 10.0$, $P < 0.0001$; Fig. 3g). Differences in bite propensity between species remained consistent when we included the body size of individuals into models (Table S1).

3.2.1. Agonistic interactions

After excluding encounters involving the single hybrid individual, we obtained results for 161 behavioural encounters (65 between two Aeolian lizards, 23 between two Italian lizards and 73 between the two species); for each individual we retained on average 5.0 encounters (on average, 2.5 as resident and 2.5 as intruder). The number of attacks strongly depended on the identity of the opponent. If the opponent was an Italian lizard, lizards attacked significantly less than when the opponent was an Aeolian lizard (Table 1, Fig. 4). Furthermore, resident lizards attacked significantly more than intruders. The species identity of the lizard did not have a significant effect on the number of attacks, but we found a significant interaction between the species identity of focal lizards and their opponents, indicating that Aeolian lizard strongly decreased attack rate when facing an Italian lizard (Fig. 4). On average, Aeolian lizards attacked Italian lizards 4.4 times less than conspecifics (Fig. 4). The model explained a good amount of variability for the number of attacks (Table 1).

We found strong differences between species for the number of escapes. Aeolian lizards escaped significantly more than Italian lizards (Fig. 4, Table 1). The number of escapes was unaffected by the species identity of the opponent, nor by the role (resident vs. intruder) of test individual. Nevertheless, a significant interaction between species identity of focal lizards and their opponent indicated that Italian lizards very rarely escaped when facing an Aeolian lizard (Table 1). In interspecific encounters, the Aeolian lizards escaped 5.0 times more than the Italian lizards.

4. Discussion

Interspecific competition is certainly a major impact of biological invasions, still the extent and mechanisms through which it can determine dramatic declines on native species remains highly debated. We show that interference competition during agonistic interactions between males can be a major process through which invasive lizards outcompete the native ones and, under strong niche overlap between the two species, this might dampen their breeding potential.

The Aeolian lizard underwent a dramatic decline during the last decades (Fig. 1), and in Vulcano only persists in a remote peninsula where geographical isolation probably limits the invasion by Italian lizards (Ficetola et al., 2021; Paris et al., 2024). Although hybridization between Aeolian and Italian lizards has been proposed as one of the major processes determining the decline (Capula, 1993), recent population genomic estimates indicate a very low hybridization rate (Fig. 2; Paris et al., 2024). Early observation of

Table 1
Results of generalized linear mixed models testing the factors affecting the number of attacks and of escapes of test lizards in agonistic interactions. R^2_M: marginal R^2.

Independent	Dependent	z	P	R^2_M
N attacks	Species of focal individual	0.538	0.591	0.36
	Species of the opponent	4.268	<0.0001	
	Role (resident / intruder)	2.446	0.015	
	Species of focal × opponent	2.408	0.016	
N escapes	Species of focal individual	5.112	<0.0001	0.43
	Species of the opponent	0.571	0.568	
	Role (resident / intruder)	0.763	0.446	
	Species of focal × opponent	2.739	0.006	

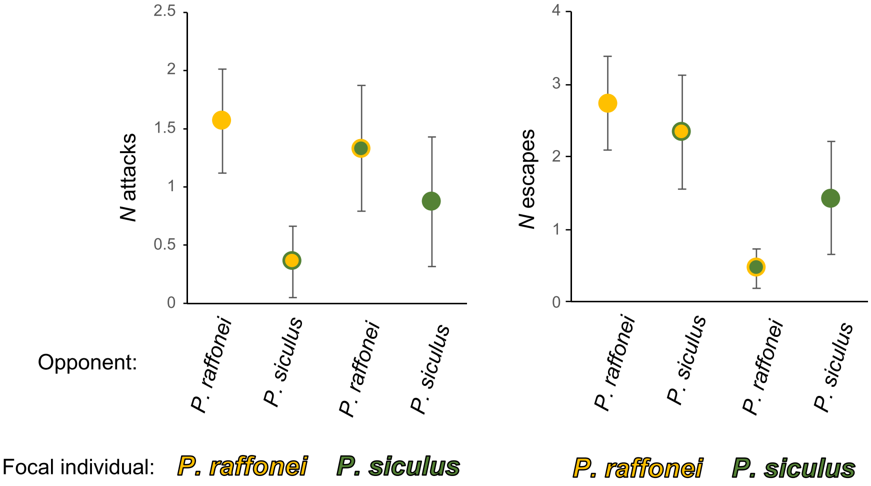

Fig. 4. Average number of attacks and escapes by endemic (*P. raffonei*) and invasive (*P. siculus*) lizards during behavioral encounters, when facing conspecifics or heterospecifics. Error bars are 2 standard errors of the mean.

atrophied gonads in F1 hybrids suggested that hybrids between these species are sterile (Capula, 1993), but rare backcrosses and lack of higher order hybrids indicate that at least some hybrids are not sterile but have very low fitness (Paris et al., 2024). This is further supported by the absence of introgressed alleles of *P. raffonei* in the genome of *P. siculus* individuals collected from other areas of Vulcano (Paris et al., 2024). Altogether these findings suggest that, even though interspecific mating and hybridization can occur, hybrid swamping is not the most likely determinant of the decline of the Aeolian lizard.

Conversely, behavioural data point out a possible role of interspecific interactions due to differences in competitive ability, mediated by both morphological and behavioural factors. First, adult Italian lizards were on average 10–20 % larger for all the considered morphometric traits (Fig. 3a-e). In lizards, body size differences are a major determinant of the outcome of agonistic interactions (Donihue et al., 2016; Sacchi et al., 2009; Scali et al., 2023). Our data confirm this pattern, with a superior competitive performance of the larger Italian lizard in staged experiments. The marked body size differences between the two species (i.e. the collinearity between body size and species) hampered testing in the same model the role of species and body size difference. If species identity was removed from models, body size difference became the major determinant of the outcome of competitive interactions (Table S1), but models with body size differences as a predictor showed a poorer explanatory power compared to the ones including species identity (much lower R^2 values), suggesting that body size alone is not enough to explain the asymmetric performance of the two species. In fact, Italian lizards were not just larger, but also showed more aggressive behaviour under a neutral stimulus (higher bite frequency and bite endurance; Fig. 3). All these factors can determine the expression of more aggressive behaviours and better performance of Italian lizards compared to Aeolian lizards.

In wild conditions, interference competition can only determine the decline of native species if niche overlap is very strong. The Italian lizard is a highly generalist species that is able to inhabit all the habitats historically inhabited by Aeolian lizards, including the natural ones, and exploit similar resources (Capula, 1992; Corti et al., 2011; D'Amico et al., 2018). A direct assessment of niche overlap between the two species would be required to conclude that species interference is the main driver of the decline of the Aeolian lizard, since anthropogenic habitat alteration can affect the interaction between Italian wall lizards and other native lizards (Mangiacotti et al., 2013). Measuring competition and niche overlap between these species in the field is at present unfeasible, as the Aeolian lizard is extinct in all the areas that have been invaded by Italian lizards. However, data from the 1980's showed that the Italian lizard can reach very high densities even in the microhabitats with natural vegetation where Aeolian lizards historically were more abundant, and recorded strong niche overlap between the two species (Pianka's overlap parameter = 0.705; Capula, 1992). Under such niche overlap, the success of Italian lizards in interspecific competition can lead to asymmetrical accessibility to key resources, with fitness consequences for the native lizards.

The outcome of interspecific encounters identified two mechanisms through which Aeolian lizards are generally outperformed. First, Italian lizards received less attacks, being almost never attacked by Aeolian lizards (Fig. 3a). Male lizards actively defend their territories from intruders, and our experimental set-up (≥3 days of acclimation) was expected to allow the establishment of territorial behaviours (Sacchi et al., 2009). Despite the role of individuals (resident vs intruder) was a significant predictor of the number of attacks, it was much less important than the identity of the opponent (Table 1). When deciding whether attacking or not, males face a trade-off between the advantage of aggressive behaviour (e.g. maintaining or acquiring a territory) and the risk of injuries. As a consequence, opponents that are perceived as stronger are rarely attacked (López and Martín, 2001). The large size of Italian lizards, and potentially additional unmeasured traits representing individual performance (e.g. chemical signals, unrecorded behavioural patterns; Scali et al., 2023) can explain the reduced rate of attacks by Aeolian lizards, which often did not even try attacking the Italian lizards even if the latter were intruders. Second, Aeolian lizards fled much more frequently. The number of escapes can be taken as a measure of defeat (i.e. is negatively related to the overall success of interactions) and indicates that interspecific interactions are usually won by Italian lizards. The relative performance of the two species can be measured as the ratio between the number of escapes of the two species, when only interspecific encounters are considered. An Aeolian lizard encountering an Italian lizard fled five times more often than an Italian lizard encountering an Aeolian one, independently from their role (resident vs. intruder) (Fig. 4). These striking differences in agonistic performances suggest that an Aeolian lizard, either if is resident in one territory or is seeking for a new one, has few chances to hold a territory following the encounter with an Italian lizard. We acknowledge that behavioural observations performed in terraria suffer several limitations, as controlled conditions are a simplification of the complexity that is found in nature (Ficetola and De Bernardi, 2005, 2006; Miner et al., 2006). Field observations would be needed to test predictions from laboratory experiments, particularly for complex processes such as interspecific interactions (Ficetola and De Bernardi, 2006; Huston, 1999; Skelly and Kiesecker, 2001). For instance, it is possible that in the field Aeolian males defend their territories more vigorously than observed in terraria (e.g. because a higher motivation despite body size difference), or that they can have a better performance under specific microhabitat conditions (discussed below). Unfortunately, such field observations currently are impossible, because the Aeolian lizards are extinct in the areas occupied by Italian lizards, and because their critically endangered status makes manipulative field experiments unfeasible as they might exacerbate its extinction risk (Ficetola et al., 2021). Future studies can use approaches such as mesocosms and comparative analyses of morphology (Dufour et al., 2018) to better assess the relative performance of the two species for multiple fitness-related parameters under a range of circumstances. Still, the few available studies from the 1980’s suggested that Italian lizards outcompete the Aeolian lizards through most of environmental conditions both in natural maquis habitats and in human-dominated habitats (Capula, 1992). Active surveillance of the contact zone between the two species can help validating the findings of laboratory experiments, and would also help the early detection and removal of Italian lizards that might invade Capo Grosso.

In nature, losing a territory implies losing the access to the associated resources and breeding opportunities. This might explain why native lizards have declined so rapidly, even though losing competition does not necessarily determine injuries or mortality (Ribeiro and Sá-Sousa, 2018). Females generally do not choose their mate, and mate with the owners of territories (Olsson and Madsen, 1998). The overperformance of invasive males in agonistic competition can determine the monopolization of territories and, in sympatry, this may severely limit the opportunity for females of Aeolian lizard to mate with conspecifics. Worryingly, the occurrence of F1 hybrids suggests some permeability in reproductive barriers between the two species, but F1 have likely low to null fitness since many of them show atrophied gonads (Capula, 1993) and very few backcrosses occur (Paris et al., 2024). Therefore, interspecific mating would result in a waste of reproductive potential. The scarcity of hybrids in present-day observations might be explained by the partial isolation of the Capo Grosso peninsula where Aeolian lizards survive (Paris et al., 2024). Indeed, few decades ago the contact zone between the two species was broader, with a higher frequency of hybrids (Capula, 1993). Moreover, females of Aeolian lizards probably lay a small number of clutches per year (Capula and Lo Cascio, 2011), thus mating with invasive males can prevent subsequent breeding, even if the female subsequently meet a conspecific male. We propose that such "sterilizing interference" might reduce the overall fitness of endemic lizards to values close to zero and provides a likely mechanism underlying the rapid decline of Aeolian lizards observed in the last decades (Capula et al., 2002; Fig. 1). This process might well explain declines of other territorial species, particularly endemic species restricted to small areas (Herrel et al., 2008).

In principle, relative performance can vary across space and, in some conditions such as refugia microhabitat, endemic lizards might have better fitness than the invasive ones (Case et al., 1994; Cole et al., 2005; Grabowski et al., 2009). Nevertheless, several experiments suggest that the Italian lizards outperform their native congeners under a range of conditions and for multiple traits (e.g. thermoregulation, food intake, parasite resistance…) (Caruso et al., 2021; Damas-Moreira et al., 2018; Damas-Moreira et al., 2020; Damas-Moreira et al., 2019; Limnios et al., 2022; Tome et al., 2021). Therefore, in natural conditions, invasive lizards might outcompete the native ones also for resource acquisition (e.g. exploitative competition for food) (Capula, 1992; Caruso et al., 2021; Damas-Moreira et al., 2020; Dufour et al., 2018; Limnios et al., 2022). Moreover, Vulcano and most Aeolian islands are small and show limited habitat heterogeneity. Recent surveys covering a range of microhabitats across multiple islands found little evidence of Aeolian lizards surviving in specific microhabitat refugia, even in the areas where Aeolian lizards were historically present and that still have natural vegetation (D’Amico et al., 2018; Muraro et al., 2022). This suggest that, even if such refugia exist, they have limited relevance for the long-term persistence of Aeolian lizards.

Considering interspecific interactions as potential driver of the shrinkage of Aeolian lizards can help planning conservation actions. Preventing the invasion by Italian lizards into the few strongholds hosting Aeolian lizards is a priority. This can be challenging, as Italian lizards have been able to invade a large number of countries (Bonardi et al., 2022; Oskyrko et al., 2022; Sabolić et al. 2024; Silva-Rocha et al., 2012; Silva-Rocha et al., 2019; Silva-Rocha et al., 2014; Tuniyev et al., 2023), and might even be able to cross narrow sea straits, for instance by rafting on vegetation (Sherpa et al., 2024). Not by chance the Italian lizard is the most widespread lizard in Mediterranean archipelagos, where it colonized almost 300 islands and islets (Bonardi et al., 2022). Aeolian lizards survive in a few tiny islets that are currently isolated from larger, invaded islands. While currently these islets are assumed to be safe for the Aeolian lizard, in the long term also these islets might receive propagules of the invasive Italian lizard. Regular monitoring is needed for early detection of eventual propagules. Furthermore, beside invasive species, the Aeolian lizard populations inhabiting these islets are very small and suffer a severely impoverished genetic diversity (Gabrielli et al., 2023; Salvi, 2023), thus they might be not enough for the persistence of the species. The preservation of these strongholds can be complemented by the identification and management of additional sites that might host further populations, without invasive lizards and thus where interference competition cannot occur. The largest islands of the Aeolian archipelago, such as Vulcano, host huge populations of Italian lizards, and complete eradication from these islands would be extremely challenging. On the other hand, management might be feasible in small, isolated sites. The success of recent captive breeding attempts suggests that such strategies can be successful, posed that they are within a long-term management plan that maintain suitable habitats and prevent future invasions. These actions are going to be implemented in recently started conservation projects (e.g. the EOLIZARD Life Project; https://cinea.ec.europa.eu/programmes/life_en).

In the last years, behaviour has increasingly been perceived pivotal to understand the interactions between native and invasive species (Weis and Sol, 2016). In territorial species, agonistic interactions between males can play a so far unappreciated role, potentially affecting breeding performance of both males and females, still field assessments measuring actual individual fitness of both sexes are required to ascertain the actual impacts of this process, and to discriminate it from alternative pathways.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: G.F. Ficetola, A. Melotto, R. Sacchi, D. Salvi reports financial support was provided by MUR. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2024.e03251.

Data Availability

Data and scripts are available on figshare at DOI:10.6084/m9.figshare.26357254

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