The role of genetic diversity in the evolution and maintenance of environmentally-cued, male alternative reproductive tactics

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Abstract

Background

Alternative reproductive tactics (ARTs) are taxonomically pervasive strategies adopted by individuals to maximize reproductive success within populations. Even for conditionally-dependent traits, consensus postulates most ARTs involve both genetic and environmental interactions (GEIs), but to date, quantifying genetic variation underlying the threshold disposing an individual to switch phenotypes in response to an environmental cue has been a difficult undertaking. Our study aims to investigate the origins and maintenance of ARTs within environmentally disparate populations of the microscopic bulb mite, *Rhizoglyphus robini*, that express 'fighter' and 'scrambler' male morphs mediated by a complex combination of environmental and genetic factors.

Results

Using never-before-published individual genetic profiling, we found all individuals across populations are highly inbred with the exception of scrambler males in stressed environments. In fact within the poor environment, scrambler males and females showed no significant difference in genetic differentiation (Fst) compared to all other comparisons, and although fighters were highly divergent from the rest of the population in both poor or rich environments (e.g., Fst, STRUCTURE), fighters demonstrated approximately three times less genetic divergence from the population in poor environments. AMOVA analyses further corroborated significant genetic differentiation across subpopulations, between morphs and sexes, and among subpopulations within each environment.

Conclusion

Our study provides new insights into the origin of ARTs in the bulb mite, highlighting the importance of GEIs: genetic correlations, epistatic interactions, and sex-specific inbreeding depression across environmental stressors. Asymmetric reproductive output, coupled with the

purging of highly inbred individuals during environmental oscillations, also facilitates genetic variation within populations, despite evidence for strong directional selection. This cryptic genetic variation also conceivably facilitates stable population persistence even in the face of spatially or temporally unstable environmental challenges. Ultimately, understanding the genetic context that maintains thresholds, even for conditionally-dependent ARTs, will enhance our understanding of within population variation and our ability to predict responses to selection.

Keywords*:*

inbreeding depression, epistasis, genetic correlation, environmental threshold model, phenotypic plasticity, conditional strategy

Background

In numerous species, it is common for individuals (usually males) to adopt different strategies to increase their reproductive success when intrasexual competition is intense. These strategies can ultimately lead to diversity within populations, comprising of characteristics such as behaviour, physiology, or morphology [1]. Referred to as alternative reproductive tactics (ARTs), strategies such as these encompass trade-offs between increased reproductive potential versus the costs incurred to produce traits under selection, often leading to the development of a less energetically demanding tactic, such as sneakers (versus guards) or satellites (versus callers). Although taxonomically widespread and studied in various organisms [1], the proximate mechanisms responsible for ART trait evolution, or the processes that maintain ARTs within single populations are not always well understood. Some ARTs are plastic by nature, driven by seemingly pure environmental effects (e.g., dung beetles, *Onthophagus acuminatus*; [2]), whereas others are fixed, determined exclusively by genetic underpinnings (e.g. lekking sandpiper, *Philomachus pugnax*; [3]), although the latter remains a relatively rarer phenomenon [4–6]. More commonly however, species demonstrating ARTs involve a combination of both genetic and environmental influences, that interrelate in genotype-by-environment interactions [7].

Genotype-by-environment interactions (GEIs) are routinely observed in traits linked to fitness [8] such that in different environments, numerous genotypes may display and switch superiority (ecological cross-over), assisting in the maintenance of variation within populations. Moreover, male sexually selected traits often show condition-dependence that is assumed to involve many loci, providing ample opportunity for mutations ('genic capture hypothesis' [9]) and genetic variation. For example, high genetic diversity (heterozygosity) has been linked to an individual's fitness and condition, including an increase in survival [10, 11], parasite resistance [12], developmental stability [13], competitive ability [14], viability

101 [9, 15, 16], mating opportunities [17], and the expression of costly secondary sexually
102 selected traits [18]. Together, GEIs and condition-linked genetic diversity may help to
103 reconcile the origin and maintenance of ARTs within populations [19], despite presumably
104 strong selective forces promoting the canalization of traits, and genetic erosion associated
105 with sexual selection ('the lek paradox') [19, 20].

106 Currently, the environmental threshold model, which links condition-dependence and
107 GEIs [21–23], is the most widely accepted process for ART expression. Specifically, this
108 model posits that environmental circumstances experienced by an individual during ontogeny
109 leads to an all-or-none response in terms of expressing ARTs, which in-turn is likely
110 influenced by the organism's genetic background [23]. Male polymorphic variation is thus
111 thought of as a threshold trait based on a continuously distributed phenotype that is
112 environmentally sensitive [24]. Threshold traits have been shown to have a heritable basis,
113 although more likely due to the heritability of the underlying threshold itself (liability traits)
114 [25, 26]. If this polymorphic variation is under polygenic control, condition-linked genetic
115 diversity likely plays an important role in trait expression. ARTs involve complex traits that
116 can be heritable, subject to selection, and evolve, yet to date, the genetic basis underlying the
117 evolution of conditionally-dependent ARTs has been difficult to quantify [27].

118 The bulb mite *(Rhizoglyphus robini)* is a microscopic agricultural pest, which thrives
119 on invading crops and disperses easily when food is deprived (a species familiar with
120 fluctuating environment conditions) [28]. This species demonstrates a short generation time,
121 has high reproductive potential [29], and is easily reared in laboratory conditions, making it
122 an ideal organism for experimental evolution studies. Intriguingly, the bulb mite demonstrates
123 a complex ART system that has recently described up to three male polymorphisms, including
124 a 'megascrambler' [30] that, due to its rareness within populations, will be excluded from the
125 current study. Of the two prominent male ARTs in *R. robini*, individuals express either a

126 'fighter' or 'scrambler' mating tactic consisting of the ontogenetic development (or not) of
127 weaponry comprised of a thickened, sharply terminated third pair of legs used to combat and
128 kill rival males (fighters and scramblers, respectively) (Fig. 1). The environmental threshold
129 model is a good candidate model to explain the evolution of this male dimorphism as high
130 nutritional quality and quantity during development increases juvenile body size, which in
131 turn increases fighter morph expression in adulthood [31, 32]. An experimental test of this
132 model's predictions on evolutionary shifts in ART expression indeed confirmed threshold
133 shifts when selecting against fighter expression. This analysis, however, failed to capture the
134 observed evolutionary threshold shifts when selecting against scrambler expression [33],
135 likely because scrambler expression shifted evolutionarily in response to the demographic
136 consequences of the experimental treatment, rather than the treatment itself. It therefore seems
137 likely that multiple environmental drivers are involved to maintain this male dimorphism
138 [34]. Previous research also demonstrates the bulb mite ART is somewhat heritable, yet these
139 heritability scores vary widely depending on population or study, ranging between 0.18 to
140 >1.00 based on experimental and modelling estimates [35–37], further suggesting this ART
141 likely does not represents a simplistic environmental or genetic trigger.
142 Here, we aim to resolve broad evolutionary questions surrounding the origin and
143 maintenance of ARTs by quantifying the cryptic variance underpinning threshold responses to
144 environmental cues. We do this by testing the hypothesis that genetic diversity differs
145 between the two male ARTs in the bulb mite such that larger fighters with associative high
146 body condition will demonstrate higher levels of genetic diversity compared to their smaller,
147 poorer conditioned scrambler counterparts. Using populations consisting of tens-of-thousands
148 of individual bulb mites reared under different environments, we quantified underlying
149 genetic context in relation to ART expression, using never before published genetic markers
150 to quantify individual-level genetic diversity across populations.

Materials & Methods

Specimen maintenance and collection

We used bulb mites from stock populations originating from 10 sampling sites via collecting flower bulbs near Anna Paulowna, North Holland, Netherlands in 2010, that ultimately comprise tens-of-thousands of individuals. Mites were reared and maintained at the Institute for Biodiversity and Ecosystem Dynamics at the University of Amsterdam, Netherlands in a controlled environmental chamber (25 ± 1 °C, 60% relative humidity, 16:8h light-dark photoperiod; sensu [38]) under two different rearing environments commonly used in life history studies to assess growth, development, and ART expression of mites from the family Acaridae (e.g., [39–41]). These two environments, henceforth be described as 'poor' or 'rich', differed only in their nutritional resources; mites were fed either rolled oats (poor food quality) or dried yeast (rich food quality via high quantity of protein), ad libitum. The rich resource treatment (yeast), in fact, creates a similar rearing environment to that of natural bulb mite populations feeding on garlic bulbs [39].

From the rich and poor environments, mites were randomly collected and examined with a stereomicroscope for identification. Sexes and ART morphs were identified according to the morphological criteria described by Smallegange [38], including size delimitation, genitalia, and the presence/absence of enlarged third leg pairs (main ART trait differentiation). Following recommendations that 20-30 individuals assayed within populations yield sufficiently reliable estimates for population genetic parameters [42], a total of 231 mites were sampled from the stock populations in both rich (n=126) and poor (n=105) environments, including 72 scrambler males (rich n=42, poor n=30), 76 fighter males (rich n=32, poor n=44), and 83 females (rich n=52, poor n=31). Upon collection, mites were

individually preserved in 1.5 mL Eppendorf tubes containing 95% ethanol, and stored at -20°C until DNA extraction.

Because we aimed to create equal sampling of each subpopulation (female, fighter, scrambler), the representative sex-ratio from the overall environments did not reflect the female biased operational sex-ratio from either stock or natural populations [33]. However, this sampling scheme, should bear no influence on our interpretation of whether genetic context influences the expression of ARTs in the bulb mite system.

DNA extraction, PCR amplification, and nSSR analysis

Prior to extraction, all ethanol within the Eppendorf tubes was evaporated. For the female, and fighter male mites, we used a modified protocol from Knegt *et al.* [43]in which chelex-based DNA extraction was performed: 4 -5 zirconium beads, 50µL of a 5% chelex solution (Bio-Rad laboratories), and 5µL of proteinase K (20 mg ml^{-1}) were added to each tube, after which the samples were homogenized 3 times for 30s at 6500 rpm using a Precellys24 tissue homogenizer (Bertin Corp). Upon homogenization, the samples were incubated for 2 hours at 56 °C, and proteinase K was inactivated via incubation for 8 minutes at 95°C. Samples were centrifuged for 2 minutes at 14000 rpm and thereafter stored at -20°C.

As scrambler male mites are typically much smaller than their fighter or female counterparts, we adjusted the DNA extraction protocol as follows: after the evaporation of all ethanol, 4.5µL proteinase K (20 mg ml^{-1}) was added to each Eppendorf tube, and with a pestle, mites were ground into small pieces, after which 30µL of a 5% chelex solution was added to each tube. The samples were subsequently incubated for 3 hours at 56 °C, and proteinase K was inactivated via incubation for 8 minutes at 95°C. Samples were vortexed and centrifuged shortly, and stored at -20°C prior to DNA amplification.

In total we tested 16 nuclear simple sequence repeats (nSSR) primer pairs designed for our species at Jagiellonian University in Kraków [44], optimizing the primer pairs and concomitant PCR protocol for our own populations using Dreamtaq polymerase (Thermo Fisher Scientific). Each primer pair was amplified individually in 15µL reactions wherein each reaction contained 3µL 5 X Dreamtaq buffer, 3µL dNTPs (10µM), 0.5µL $MgCl_2$, 0.5µL BSA, forward and reverse primers (see Table 1 for concentrations), and 0.125µL Dreamtaq polymerase. Prior to adding DNA template, DNA samples were briefly vortexed and spun-down to separate the DNA solution from the chelex beads. To each sample, 2µL of DNA template was added. The thermal cycle protocol started at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at either 51 °C or 53 °C (see Table 1) for 90 s, extension at 72°C for 90 s, and a final extension at 72 °C for 10 minutes. PCR products were stored at 7 °C until analysis (within 1 week of extraction). Samples were visually inspected using 2% agarose gel electrophoresis before fragmentation analysis.

Primer pairs were labelled with four different fluorescent tags, allowing them to be multiplexed and analysed simultaneously using capillary electrophoresis (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems). Per two amplicons (differently labelled), 1µL of PCR product, 0.3µL of orange dye labelled GeneScan™ size standard 500LIZ™ (Applied Biosystems), and 10µL formamide was added and denatured before running on the ABI analyzer. Data was visualised, and alleles were scored, in GeneMapper™ software (v4.1.1) (Applied Biosystems), after which each automatically scored allele was double-checked by hand. Our nSSRs were defined by a characteristic stutter followed by a peak of at least 450 relative fluorescent units or greater. We further assayed approximately 10% of our samples a second time to check and ensure repeatability of scoring.

Statistical analyses

With the use of GenoDive v.2.28 [45] that accounts for information gaps by drawing random alleles from the baseline allele frequencies (e.g., missing or null alleles, ensuring no individuals were excluded from analysis), various metrics of genetic diversity were calculated. Beyond calculating the number of alleles per locus (n_A), we also quantified observed heterozygotes within a subpopulation (i.e., females, fighters, scramblers) (H_O) and expected frequency of heterozygotes (H_S) under Hardy-Weinberg equilibrium (HWE) [46], both ranging from 0 to 1. These metrics were then used to calculate the inbreeding coefficient (G_{IS}), and determined whether subpopulations departed from HWE (ranging from -1, more heterozygosity than expected, to 1, less heterozygosity than expected). To measure genetic divergence among subpopulations, Wright's F_{ST} was estimated according to F-statistics defined by Weir & Cockerham [47], whereby the ratio of heterozygosity within the subpopulation is compared to the total population (ranges from 0 - little to no genetic divergence between populations, to 1 - total divergence between subpopulations).

We also performed an analysis of molecular variance (AMOVA) [48] in GenoDive to test for population genetic structure; calculations were performed on four different hierarchical levels (between environments [rich and poor], between subpopulations [sexes and morphs] within environment, among individuals within subpopulations, and within individuals), and gives us insight in the genetic differentiation between these different levels. Statistical significance was evaluated based on 999 random permutations and distances were calculated using the Infinite Alleles Model.

We further subsampled 30 random individuals per group and performed the same analyses with the aim to control for possible artefacts or bias within our analyses stemming from missing data or unequal sampling. Random subsampling and reanalysis was performed 5 times (exemplar represented in Additional File 2, Table S2.1-S2.5).

STRUCTURE analysis (GenoDive v2.28 [45]; STRUCTURE add-in [49]) was additioanlly used to infer genetic clustering using the multilocus nSSR data within populations (rich and poor environment) among the respective subpopulations (i.e. females, scramblers and fighters). This analysis used a Monte Carlo Markov Chain (MCMC) to identify genetically distinct clusters by assigning individuals to K clusters based on assignment probability (Q-value), minimizing departures from HWEand linkage equilibrium. We used a 5×10^3 burn-in, followed by 5×10^4 iterations assuming admixture and correlated allele frequencies without prior population information. We ran 1 to 10 K clusters, with 20 replicates for each cluster. Optimal population clusters were determined according to delta K [50] and bar plot visualisations were compiled using the program STRUCTURE PLOT [51].

Results

After protocol optimization, we found only 9 of the 16 nSSRs amplified well for our populations, of which 3 loci revealed fixation, and 6 demonstrating both clean/readable peaks and polymorphism across individuals. Thus, these 6 nSSRs were chosen for the genotyping of all remaining individuals.

Across individuals, we had a total of 12.3% missing or null alleles; 3.6% in females, 12.8% in males (25.9% in fighters, 3.7% in scramblers). In the poor environment (19.1%), missing data for females was 5.4%, and for males, 20.5% (34.5% in fighters, and 0.00% in scramblers). In the rich environment (10.2%), missing data for females was 8.65%, and for males, 9.68% (14.1% in fighters, and 6.4% in scramblers). We additionally detected 11 private alleles across 5 loci that differentiated between males and females, and 12 alleles that segregate between the rich (4) or poor (8) environments (Additional File 1, Table S1).

Across all individuals, allelic richness remained low, ranging from 3 to 11 alleles per locus. For four loci, significant deviations from HWE were detected demonstrating an excess

of homozygosity present across individuals (Table 1). Deviations from HWE were also detected within our rich and poor environments (Table 2), where rich environments contained significantly lower levels of heterozygosity across all individuals compared to expectation. Poor environments similarly demonstrated lower than expected levels of heterozygosity across all individuals, with the exception of scramblers that were shown to not significantly differ from expectation. These patterns also corresponded to significant levels of inbreeding (G_{IS}), with the exception of scramblers in the poor environment.

Pairwise genetic differentiation between environments (rich and poor) differed significantly (F_{ST} = 0.109, p<0.001) between the subpopulations (female, fighter and scrambler) within their respective environments (Table 3), with the exception of scramblers compared to females in the poor environment. Although fighters and scramblers significantly differed from each other within both environments (rich and poor), genetic differentiation was approximately three times lower in the poor environment compared to the rich environment (F_{ST} = 0.036, p<0.001, and F_{ST} = 0.102, p<0.001, respectively). These results corroborate the findings that fighters were significantly more genetically divergent compared to scramblers within either environment (Fig. 2).

AMOVA analysis (Table 4) showed significant genetic differentiation across subpopulations (females, fighters, and scramblers; F_{SC} = 0.085, p<0.001), between morphs (fighters, and scramblers; F_{SC} = 0.073, p<0.001), and between sexes (females, males; F_{SC} = 0.069, p<0.001). Subpopulations within environment were also genetically different from one another (F_{SC} = 0.085, p<0.001), but the environments (rich and poor) do not differ from the total population (F_{CT} = 0.083, p = 0.206).

With the exception of locus Rrms 72 demonstrating no significant deviations from HWE (Additional File 2), our subsampled analyses demonstrated near identical results in

297 accordance with our original data set, suggesting any missing/null alleles and unequal
298 sampling within our populations had negligible impact on our results.

299 Our STRUCTURE analysis demonstrated 2 genetic clusters based on delta K [50]
300 (K=2) best fit our data. Genetic clustering similarly illustrated females and scramblers to
301 disproportionately cluster together compared to fighter individuals that formed their own
302 genetic cluster, although this pattern was more stark in rich compared to poor environments
303 (Fig. 3).

304

305 ## Discussion

306 Despite previous formative work focusing on sex- and morph-specific population mean
307 transcriptome patterns in *R. robini* [52, 53], this study is the first of its kind to quantify
308 individual-level genetic diversity in the bulb mite, building a foundation for further genetic
309 quantification investigations for this microscopic organism. Importantly, due to this
310 individual-level approach, the results from this study demonstrate that ARTs in the bulb mite
311 system are associated with genetic diversity, which in-turn is further connected with
312 environmental effects (GEIs). The finding that GEIs underlie the pattern of ARTs is likely to
313 have important repercussions to our understanding of selection in this species, and may help
314 to resolve the previously (but confined) observations for genetic (e.g., [52, 53]) and
315 environmental (e.g., [33, 35, 54]) components operating to mediate male trait expression.
316 GEIs may further help to explain how this polymorphism is maintained within populations
317 over time, notwithstanding often disparate and fluctuating environmental challenges.

318

319 *GEIs, genetic context, and the origin of bulb mite ARTs*

320 Counter to our hypothesis for genetic diversity-condition links within our male morphs, we
321 find evidence that large fighters are less genetically variable than their smaller scrambler

322 counterparts. As fighters have been shown to achieve higher reproductive success than
323 scramblers [55], while also being capable of killing conspecifics within populations [56, 57],
324 it is not entirely surprising that these individuals are less genetically diverse simply as a by-
325 product of effective population size reduction [58], and thus genetic erosion. Indeed, both
326 mating monopolization and increased survival likely combine to effectively limit the genetic
327 pool in ensuing fighter offspring. Alternatively, sex-specific effects of inbreeding depression
328 on fitness are also plausible [59], especially in light of high inbreeding consequences on
329 female bulb mite fecundity in general [31]. Previous studies have proposed that the fitness
330 decline of *R. robini* females derived from fighter selection-lines is evidence for intralocus
331 sexual conflict [60]. Our observations that fighters are more inbred than scrambler males,
332 could equally imply that inbred females are less fit and have a higher probability of being
333 purged within populations, similar to life-span and mortality patterns observed in another
334 invertebrate with sex-specific inbreeding depression [61].

335 A non-mutually exclusive but more adaptive explanation for the origins of the genetic
336 patterns underlying bulb mite ART expression could be their genetic context, or the relation
337 and interaction of genes underpinning this phenotype (epistasis or genetic correlation). Non-
338 additive, epistatic combinations [62, 63] are likely more important than individual genetic
339 components, with pervasive effects from selection to speciation [64]. These genetic
340 interactions have also previously been shown to influence complex traits [65], alter
341 evolutionary trajectories of phenotypes [66], and underlie missing heritability [67].

342 In the bulb mite specifically, *positive epistasis* could be responsible for fighter
343 expression, such that many alleles in conjunction work in a way that synergistically
344 outperforms their individual contributions to genetically determine the fighter phenotype.
345 Similarly, if many alleles in coordination lead to a less fit phenotype than expected based on
346 their effects in singularity, the process may give rise to a new/alternative phenotype within a

347 population; certain genetic elements combinations may also mask the effects of others
348 (antagonistic epistasis), functionally suppressing the manifestation of high fitness traits (e.g.,
349 [68]). The last two aforementioned processes of *negative epistasis* could conceivably produce
350 scramblers within our populations.

351 Correspondingly, genetic correlations among traits could equally link genetic
352 components together causing similar patterns to the ones we see here. ART-specific genetic
353 correlations have been previously shown in another invertebrate taxa [69], and the breakdown
354 of co-adaptive gene-complexes has been implicated in the adoption of a flexible condition-
355 dependent ART [70], together suggesting that genetic context may be a pervasive, important,
356 but under-investigated facet to ART research. Indeed, markedly distinct genetic patterns
357 among ARTs may be expected owing to the correlational selection for various trait optima
358 combinations between morphs. Ultimately, this correlational selection will result in linkage
359 disequilibrium (opposed and eroded by recombination) having far-reaching evolutionary
360 consequences such as the loss of genetic variation, especially for species frequently
361 undergoing genetic drift through founder effects [7].

362 Insomuch as complex gene-network for traits are presumed ubiquitous [71], and
363 pleiotropic effects in a single locus for systems necessary to support multi-faceted plasticity
364 (e.g., in morphology, physiology, behaviour) seems dubious [7], it's likely that heterozygosity
365 in the bulb mite breaks apart genetic elements that require the coordination for the expression
366 of the fighter phenotype, such as specialized developmental trajectories, large body size,
367 aggression, and weaponry. Accordingly, the threshold for fighter development may require a
368 reduction to heterozygosity, such that when heterozygosity within populations decreases, the
369 threshold for fighter expression concomitantly also decreases. Threshold shifts as a response
370 to ART relative fitness would then reflect cryptic genetic variation underlying the translation
371 of the environmental cue to phenotype in a condition-genotype coupling [27]. Future *R. robini*

work should aim to assess whether these same GEI patterns are also reflected in natural populations. However, as these broad GEI associations remain consistent between rearing environments, and our rich environment reflects similar natural history responses to that of natural resources (e.g., garlic bulbs [39]), we have no reason to believe that stock and natural populations would differ in their overall patterns of ART genetic context.

Population-level diversity and the maintenance of ARTs

Considerable variation has been observed in the effects and strength of inbreeding depression, among environments, populations of the same species, and even within sexes (e.g., [61, 72–74]). Our study demonstrates that bulb mites generally lack genetic diversity across individuals, but this pattern could stem from a number of scenarios. For example, in our investigation, near even numbers of scrambler, fighter, and females were collected and compared, yet in reality (stock and wild populations), operational sex-ratios are female skewed ([33, 57], pers. observation), and ART frequencies fluctuate within populations based on environmental milieu [54]. In effect, the average genetic contribution of fighters both within poor and rich environments, compared to the combined contribution of scramblers and females, is likely highly over-represented. Moreover, lab reared populations are known to undergo genetic drift and demonstrate lower than average genetic diversity compared to their wild counterparts [75–77]. However, similar to other species [73], bulb mites may also display a general lack of inbreeding consequences. That said, the combined evidence that fighter phenotypes achieve higher reproductive success than scramblers [55], and that bulb mite ARTs demonstrate some level of heritability [35–37] but no frequency-dependence [34, 55], has continuously raised questions as to how these male polymorphisms are sustained within populations. Certainly the added evidence that fighter phenotypes are also associated with excess homozygosity (this study) further complicates our understanding of how male

phenotypic and genetic variation are sustained in this system. Here we link the genetic architecture and life-history parameters of ARTs with oscillating environmental conditions, and suggest that these evolutionary-ecological dynamics may hold the answer.

Previous empirical evidence in bulb mites not only demonstrates that scrambler morphs live longer [78], but importantly, that scrambler-selected lines produce more females that lay larger and more eggs over a longer period of time [79], and are generally more fecund than fighter-selected lines [60]. These morph-specific patterns may help to elucidate why we observed the genetic architecture of scramblers and females to be more similar to each other in contrast to fighters, patterns corroborated in gene expression profiles [52]. Similarly, these reproductive patterns may also help explain how fluctuating environmental conditions, and thus the ensuing shifts in ART frequencies, assist in maintaining genetic diversity within this species. For example, individuals that accumulated deleterious mutations otherwise buffered in optimal conditions (e.g., fighters and possibly female offspring of fighters in the bulb mite) would eventually be purged within poor (presumably stressful) environments (e.g., [74]). This mutation-selection balance could also reduce the genetic differentiation between morphs and sexes, as seen in our bulb mite individuals raised in the poor environment. Certainly, genetic variation in the threshold underlying sensitivity to environmental cues, as assumed in the environmental threshold model [21, 22], would thus cause genetic, and therefore concomitant demographic, oscillation within populations, conceivably facilitating stable population persistence even in the face of spatially or temporally unstable environmental challenges.

Across taxa, processes for the maintenance of genetic diversity are especially significant as they serve as a means for populations to adapt to changing environments and thus play an important role for the survival of a species [80], including reducing its vulnerability to ecological challenges, such as disease or climate change [81]. Whether ARTs buffer populations from excessive inbreeding, and are more likely to evolve in species that

routinely encounter boom-bust cycles or environmental perturbations, is certainly a worthy future investigation.

Conclusion

The complexity, and need for organisms to interact with their environment (to adjust, acclimatize, development, and maximize fitness) implies that genetic context, and thus GEIs, are likely to be pervasive even among plastic phenotypes. Still, the evolution and proximate cause of these phenotypic alternatives are only beginning to be understood. Ultimately, our ability to accurately predict responses to selection based on the genetic variation that maintain thresholds for ARTs, and appreciating the relative genetic and environmental contributions influencing phenotypic expression, is critical to understanding both the breadth and maintenance of within-species variation and a populations capacity to adapt to external adjustments.

Declarations

Ethics Approval: In accordance with Dutch law, no ethics approval is required for work conducted on *Rhizoglyphus robini*.

Consent for Publication: not applicable

Availability of Data and Material: The datasets generated and/or analysed during the current study are available in the Dryad repository (uploaded upon acceptance).

Competing Interests: Authors declare no competing interests

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Authors' Contribution: KAS designed the experiment, MRK designed the primers, and RD performed the genetic amplifications and analysis. KAS and RD wrote the majority of the

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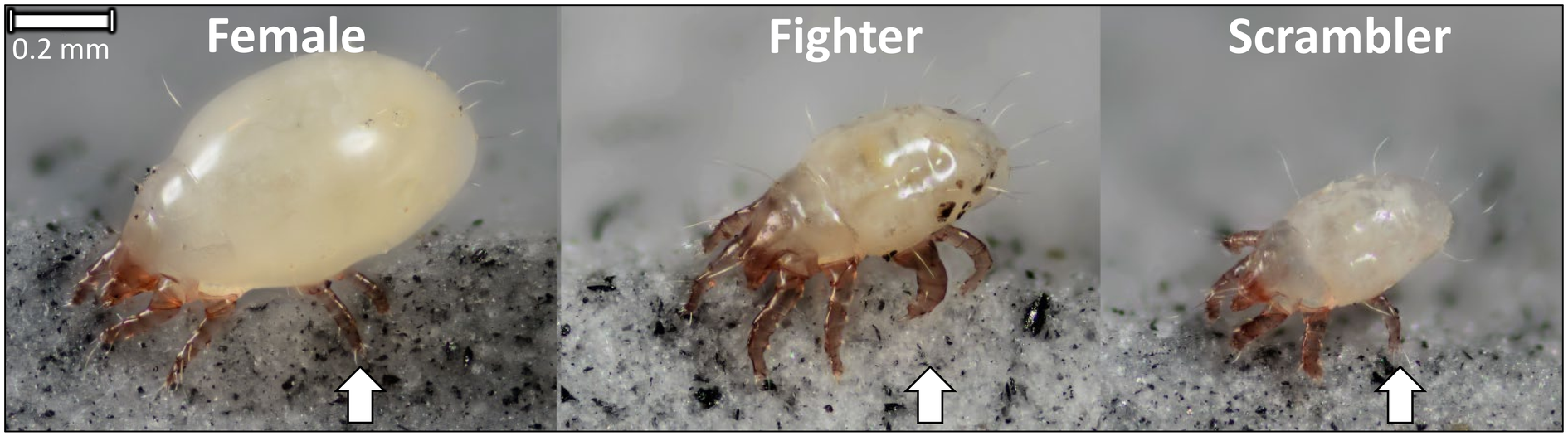
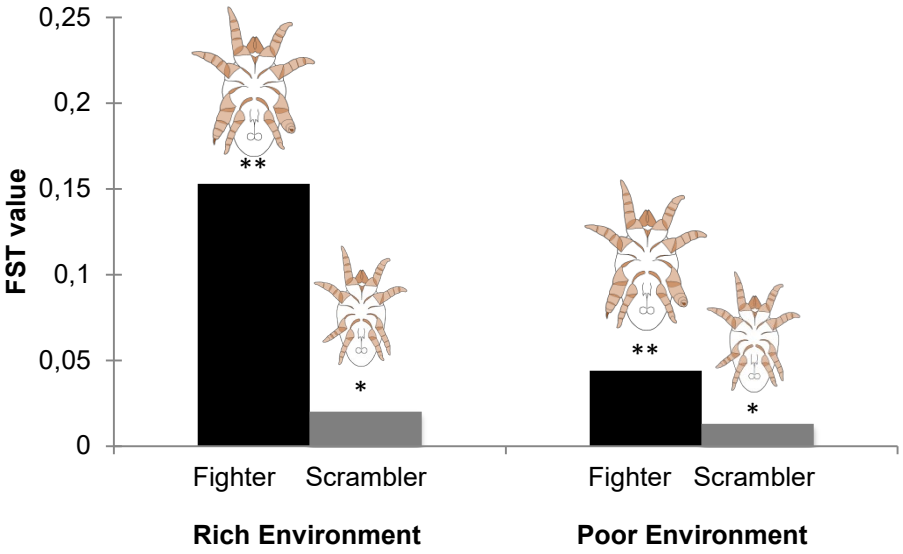
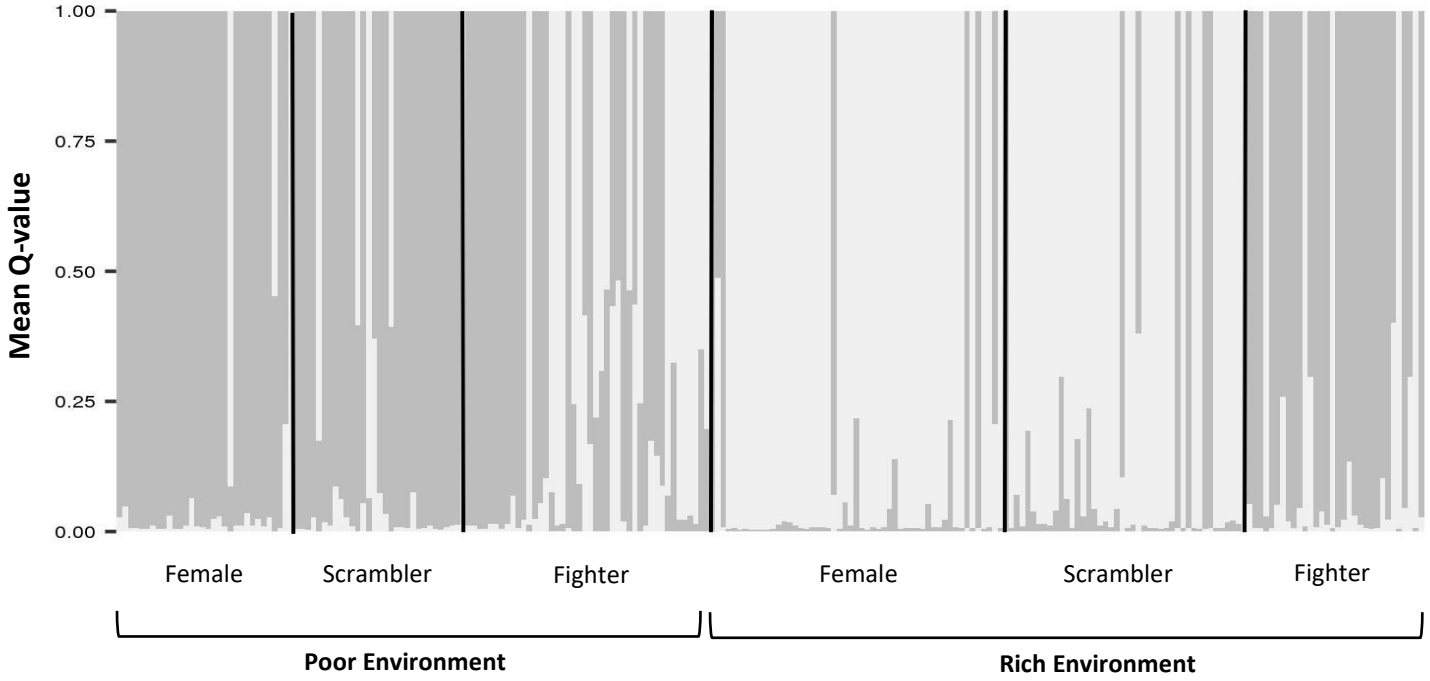
Table & Figure Captions

671 **Table 1. nSSR summary information on each locus.** Names, type of repetitive motif, size
672 range of alleles (bp), primer sequences (forward - F, reverse - R), number of alleles (n_A),
673 annealing temperature (T_a), primer concentration used in PCR amplification (Pc), and
674 observed (H_O) and expected (H_S) heterozygosities, with corresponding p-values.
675 **Table 2. Hardy-Weinberg statistics across environments and subpopulation.** Shown are
676 observed (H_O) and expected (H_S) heterozygosities, inbreeding coefficient (G_{IS}) according to
677 Nei's statistics (1987), and p-value.
678 **Table 3. Pairwise F_{ST} values for population differentiation.** Shown are the genetic
679 differentiation values per subpopulation, Poor (P), and Rich (R) environments, Female (F),
680 Male Fighter (MF), and Male Scrambler (MS) subpopulations. Significant differences are
681 represented by * after Bonferroni correction.
682 **Table 4. Summary of hierarchical AMOVA.** AMOVA including standard deviation (jack-
683 knifing over loci), % of variation, and values of the F-statistic on different levels (between
684 environment, among subpopulation within environment, among individuals within
685 subpopulation, and within individuals), with their corresponding F and p-values. F_{CT} = the
686 proportion of total variance that results from genetic differences among groups, F_{SC} = the
687 proportion of variance among subpopulations within clusters, F_{IS} = the proportion of variance
688 among individuals within subpopulation, F_{IT} = the proportion of variance among individuals
689 within the total population**.**
690 **Figure 1. Dorsolateral photographic images of adult bulb mites (*Rhizoglyphus robini*)**
691 **including the female, and male ARTs (fighter, and scrambler).** All individuals are
692 presented at the same scale (scale bar: top left) and aligned from largest to smallest (left to
693 right), with arrows indicating major structural differences in the third-leg pair among sexes
694 and morphs. Photographs produced by Jan van Arkel, 2017.

695 **Figure 2. Genetic differentiation (F_{ST}) of ART strategies to total population within each**
696 **environment.** Significant differences are represented above bars, * $p<0.05$, ** $p<0.001$. ART
697 images kindly supplied by F.T. Rhebergen.
698 **Figure 3. STRUCTURE plot of subpopulation genetic clusters in different**
699 **environments.** STRUCTURE plot illustrating the mean proportional membership (Q-value)
700 of *R. robini* individuals (females, scramblers, fighters) for K=2 across poor and rich
701 environments.
702






Mean Q-value
1.00
0.75
0.50
0.25
0.00
Female
Scrambler
Fighter
Female
Scrambler
Fighter
Poor Environment
Rich Environment

Tables

Table 1.

Locus	Nucleotide repeat	Size (bp)	Primer sequence	n_A	T_a (°C)	Pc (μM)	H_O	H_S	p-value
Rrms18	CATT	130 – 143	F: GCTTTCATTGTTGTACACCTC R: ACAAACAGCAATGAGGTACAG	4	53	3	0.171	0.488	<0.001
Rrms34	TGAA	106 – 136	F: AATAATGTTTCGCACTGAGAG R: CAAGGTAGACCGTTACAGTGA	11	53	15	0.748	0.772	0.183
Rrms40	CACT	85 – 118	F: GTAATGGCCATGTCACTAGC R: TTTGAGACTCGAAAGAAACAG	9	53	10	0.246	0.577	<0.001
Rrms44	GAGT	91 – 98	F: CTATGTTGAAAAGGCATCAAT R: GCAAAGTGTTGTTCACTCAAT	3	51	15	0.438	0.404	0.108
Rrms72	CATT	128 – 142	F: GAAATGTCAAAGACGAAAGTG R: TTGAAGTGCGAAATTAGTCAT	8	51	15	0.707	0.711	<0.05
Rrms91	GAGT	84 – 92	F: CTATGTTGAAAAGGCATCAAT R: GCAAAGTGTTGTTCACTCAAT	4	51	5	0.587	0.625	<0.001
Rrms03	AATA	147 – 149	F: AACTTGGTCTAAAGTGAAGCA R: TTGAAAAGTCACTAAGCCAAC	2	53	5	-	-	-
Rrms23	CTCC	141 – 142	F: CCGTAATGTACGACAAAGTGT R: AAGGTAATCTATCCCCCACT	2	53	15	-	-	-
Rrms61	CGA	74 – 76	F: TAAATAGATCGAGACGACCAA R: TCTCTGTGTGAACGATCTGTA	2	53	15	-	-	-

Table 2.

	Subpopulation	H_O	H_S	G_{IS}	p-value
Poor		0.487	0.578	0.158	<0.001
	Females	0.457	0.520	0.120	<0.05
	Fighters	0.390	0.566	0.311	<0.001
	Scramblers	0.583	0.603	0.032	0.307
Rich		0.398	0.600	0.336	<0.001
	Females	0.362	0.501	0.277	<0.001
	Fighter	0.329	0.581	0.436	<0.001
	Scramblers	0.479	0.605	0.208	<0.001

Table 3.

	P_F	P_MF	P_MS	R_F	R_MF	R_MS
P_F	--					
P_MF	0.063*	--				
P_MS	0.024	0.036*	--			
R_F	0.269*	0.147*	0.214*	--		
R_MF	0.085*	0.103*	0.109*	0.213*	--	
R_MS	0.178*	0.082*	0.131*	0.054*	0.102*	--

Table 4.

Variance component	SD	Variation (%)	Statistic	F-value	p-value
Between environment	0.036	0.083	F_{CT}	0.083	0.206
Among subpopulations in environment	0.038	0.078	F_{SC}	0.085	<0.001
Among individuals in subpopulation	0.113	0.184	F_{IS}	0.219	<0.001
Within individuals	0.120	0.655	F_{IT}	0.345	<0.001