



When lizards shift to a more plant-based lifestyle: The macroevolution of mutualistic lizard-plant-interactions (Squamata: Sauria/Lacertilia)

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ABSTRACT

Pollination and seed dispersal of plants by animals are key mutualistic processes for the conservation of plant diversity and ecosystem functioning. Although different animals frequently act as pollinators or seed dispersers, some species can provide both functions, so-called 'double mutualists', suggesting that the evolution of pollination and seed dispersal may be linked. Here, we assess the macroevolution of mutualistic behaviours in lizards (Lacertilia) by applying comparative methods to a phylogeny comprising 2,838 species. We found that both flower visitation (potential pollination; recorded in 64 species [2.3% of total] across 9 families) and seed dispersal (recorded in 382 species [13.5% of total] across 26 families) have evolved repeatedly in Lacertilia. Furthermore, we found that seed dispersal activity pre-dated flower visitation and that the evolution of seed dispersal activity and flower visitation was correlated, illustrating a potential evolutionary mechanism behind the emergence of double mutualisms. Finally, we provide evidence that lineages with flower visitation or seed dispersal activity have higher diversification rates than lineages lacking these behaviours. Our study illustrates the repeated innovation of (double) mutualisms across Lacertilia and we argue that island settings may provide the ecological conditions under which (double) mutualisms persist over macroevolutionary timescales.

1. Introduction

The role of species interactions in macroevolution has been acknowledged since Darwin's book 'On the Origin of Species' (Darwin, 1859), in which he provided ample examples of competition and predation driving evolutionary change and lineage diversification. Although his later work on orchids (Darwin, 1862) suggested that mutualistic interactions were probably important for diversification as well, mutualisms as drivers of macroevolution have long been neglected (Hembry and Weber, 2020). Work by e.g. Grant and Grant (1965) and Stebbins (1970) raised again attention to mutualistic interactions between plants and animals as an important driver of evolution. Since then, many cases of pollinator-driven evolution have been documented, including cases of coevolution, where plant and pollinator evolve reciprocally, e.g. yucca and yucca moths (*Tegeticula*, *Parategeticula*; Godsoe et al., 2008), figs and fig wasps (Agaonidae, Chalcidoidea; Borges, 2015; Cruaud et al., 2012; Su et al., 2022), and leafflower trees (*Glochidion*) and leafflower moths (*Epicephala*; (Hembry et al., 2013).

Similarly, periods of rapid coevolution between plants and fruit-eating and seed-dispersing animals (frugivores) have probably occurred numerous times in evolutionary history (Eriksson, 2016), with strong effects on co-evolving traits (Onstein et al., 2022, 2020; Valenta and Nevo, 2020). Although animal-plant-mutualisms often affect trait evolution and diversification in plants (e.g. Ayasse et al., 2011; Davis et al., 2014; Lagomarsino et al., 2016; Tripp and Tsai, 2017; van der Niet et al., 2014; van der Niet and Johnson, 2012; Wang et al., 2021, Onstein et al., 2022; 2020) and their animal interaction partners (Gómez and Verdú, 2012; Schweizer et al., 2014; Su et al., 2022), it is still under debate whether mutualisms increase diversification (Lengyel et al., 2009; Onstein et al., 2017; Weber and Agrawal, 2014) or rather restrict it (Smith et al., 2008; Yoder and Nuismer, 2010).

Mutualisms are also essential for ecosystem functioning (Chomicki et al., 2020, 2019). For example, animals may pollinate flowers and/or disperse the seeds of plants, and thus contribute to plant reproductive success, while benefiting from floral rewards (e.g. nectar or pollen) or essential nutrients from fruits or sap. Interestingly, some animals may

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act as both pollinator and seed disperser of the same plant, i.e. as ‘double mutualists’. The term was first coined for a lizard-plant-system in which the Mauritian endemic plant *Roussea simplex* was found to depend on the gecko *Phelsuma cepedianana* for pollination and seed dispersal (Hansen and Müller, 2009). However, double mutualisms may be generally rare as they increase vulnerability to perturbation and loss of an interaction partner (Fuster et al., 2019). This is because the loss of the animal interaction partner, for example due to environmental changes, will have a twofold negative impact on the plant’s fitness by reducing seed set (reproduction) and dispersal (survival), ultimately increasing the extinction risk of the plant and instability of the ecological community in which it is embedded. Conversely, double mutualisms may evolve more frequently when there is a scarcity of interaction partners due to natural or anthropogenic disturbances (Fuster et al., 2019). Not only are double mutualisms associated with a higher risk for the plant, but the difference in timing of the plant providing floral rewards (nectar, pollen) and fruits also requires the animal interaction partner to return to the plant during different time periods. This is associated with additional energy expenditure (e.g. climbing on trees or searching for inconspicuous flowers), which may not be favoured if other, more rewarding food resources (e.g. insects) are available. The specific ecological and evolutionary determinants for the evolution and persistence of double mutualisms therefore remain enigmatic (Whitaker, 1987).

It is well known that birds, mammals and insects interact with plants in a mutualistic fashion. However, the role of reptiles in plant pollination and seed dispersal has long been considered to be minimal due to their often carnivorous life style (Cooper and Vitt, 2002; Olesen and Valido, 2003). Recently, the importance of lizards (Lacertilia) as potential seed dispersers (Olesen and Valido, 2004, 2003; Valido and Olesen, 2019, 2007) and flower visitors/pollinators (Chamorro et al., 2012; Cozien et al., 2019; Olesen and Valido, 2004, 2003; Whitaker, 1987) has gained more evidence. Indeed, an increasing number of Lacertilia has been described as seed dispersers (see Valido and Olesen 2019 for a comprehensive review) but evidence for flower visitation is still very scattered and comparatively rare (but see Correcher et al., 2023). Interestingly, islands seem to harbour more lizard-plant-interactions than any other place, probably because islands often show high lizard densities due to reduced predation pressures, and a lack of insects, which in turn leads to a shortage of pollinators for plants and a shortage of food resources for lizards (Olesen and Valido, 2003). Hence, many island lizards supplement their diet with fruits and/or nectar from plants (Olesen and Valido, 2003; Valido and Olesen, 2019). While drinking nectar, pollen may attach to the snout and/or head of the reptile, which can then be transported to the next plant to potentially pollinate it. Similarly, feeding on fruits facilitates swallowing and subsequent dispersal of seeds. Considering frugivory and potential seed dispersal, Valido and Olesen (2019) showed that frugivory in Lacertilia is also positively correlated with insularity. This suggests that island conditions may have selected for the evolution of flower visitation, pollination and seed dispersal in Lacertilia, and thus the evolution of double mutualisms, but this has never been evaluated using a quantitative, phylogenetic comparative approach.

Here, we use a macroevolutionary framework to test the hypothesis that the evolution of flower visitation/pollination and seed dispersal by Lacertilia is linked, and could explain the emergence of double mutualisms. Specifically, we predict (P1) several independent and repeated evolutionary origins of mutualisms with plants (flower visitation, seed dispersal) across the Lacertilia Tree-of-Life, and that the evolution of seed dispersal activity pre-dates flower visitation/pollination, because flower visitation seems less common than seed dispersal in Lacertilia (see Correcher et al., 2023; Valido and Olesen, 2019). Moreover, we predict (P2) that seed dispersal activity and flower visitation are evolutionary correlated, suggesting that the evolution of seed dispersal may have provided the ecological and evolutionary setting –lizards visiting plants– for the subsequent evolution of flower visitation and pollination. Correlated evolution therefore provides a starting point to

further explore the eco-evolutionary mechanisms underlying the evolution and distribution of double mutualisms. Finally, we predict (P3) that the evolution of flower visitation and seed dispersal behaviour has influenced diversification rates in Lacertilia, because both behaviours are ecologically relevant, particularly in (insect) scarce environments (see above). Mutualistic behaviours may have provided an evolutionary advantage and therefore the ecological opportunities for increased speciation and/or decreased extinction rates in mutualistic lineages compared to lineages lacking these behaviours.

2. Material and methods

2.1. Data collection and literature search

To obtain data on flower visitation and potential pollination interactions, we conducted a literature search with Google Scholar (last search February 2021) using the search terms ‘lizard’ OR ‘reptile’ AND ‘pollinat*’ OR ‘pollen’ OR ‘nectar’ OR ‘flower visit*’. In terms of flower visitation, an interaction between a lizard and a plant was included in the final dataset (Supplementary Table S1) if it was considered to be mutualistic and would potentially contribute to pollination, i.e. cases of flower consumption (herbivory) or extra-floral nectar consumption (two cases) were discarded. Moreover, we also recorded in which cases flower visitation may lead to pollination. In order to characterise as pollinator, a lizard would have to (1) visit a flower, (2) get pollen attached to its body and (3) transport the attached pollen to the stigma of another flower of the same species. As most studies did not specifically investigate all three criteria and to avoid a severe underestimation of potential plant-lizard pollination interactions, we used the following criteria to characterize an interaction as ‘potential pollination’: (1) the lizard was observed to visit a plant and thereafter carry pollen on its body or (2) the lizard was covered in pollen of plants it had supposedly visited, and potentially pollinated. For all ‘potential pollination’ interactions we also evaluated whether the lizard has been suggested or described as a primary pollinator of at least one of the plant species it visited. Data on frugivory by Lacertilia were taken from Valido and Olesen, (2019). Although we are aware that frugivory will not always result in successful seed dispersal, we considered all recorded frugivorous Lacertilia as potential seed dispersers since multiple studies showed that frugivory by reptiles often results in the dispersal of viable seeds (Castilla, 2000; Martín-García et al., 2005; Rodríguez-Pérez et al., 2005; Traveset, 1998; Valido and Nogales, 1994). We did not consider the relative importance of nectar or fruit in the diet of the Lacertilia species (i.e. the degree of frugivory) because to infer the (correlated) evolution of those behaviours, the presence of each of those behaviours, and thus the potential to act as seed disperser/flower visitor, is the critical aspect. Moreover, throughout this study we will refer to a Lacertilia species as a ‘double mutualist’ if it has been recorded as a flower visitor and a seed disperser of the same or different plant species. This differs from the ‘classic’ definition of a double mutualism, which refers to pollination and seed dispersal of the same plant species (Hansen and Müller, 2009). However, due to the lack of data on flower visitation and pollination interactions, we argue that several of our potential ‘double mutualists’ will also qualify as double mutualists in the ‘classic’ sense when more data on lizard-plant interactions become available.

2.2. Phylogenetic reconstruction

To reconstruct the evolution of flower visitation and seed dispersal by Lacertilia, and to test for correlated evolution of these mutualistic services, we used the phylogeny by Zheng and Wiens (2016), excluding all but two outgroups (*Sphenodon punctatus*, *Alligator mississippiensis*) as well as snakes (Serpentes) and worm lizards (Amphisbaenia). We also added eight Lacertilia species that were missing from this phylogeny, but are known to interact with plants. One of those species (*Pseudocordylus subviridis*) was placed onto the phylogeny solely based on

taxonomic information due to a lack of sequence data. For the other seven species (*Bavayia robusta*, *Microlophus jacobii*, *Microlophus indefatigabilis*, *Niveoscincus microlepidotus*, *Tropidurus semitaeniatus*, *Leiocephalus inaguae*, *Tropidosaura essexi*) we inferred their phylogenetic position using RAXML v. 8.2.12 (Stamatakis, 2014). For this, we extracted all available gene sequence data from NCBI and realigned each of the 14 genes (two mitochondrial ribosomal, three mitochondrial protein-coding and nine nuclear protein-coding genes) with the sequence alignments provided by Zheng and Wiens (2016). Protein-coding genes were realigned using the translation alignment algorithm implemented in Geneious v. 7.0.6 (Kearse et al., 2012) with the default (Blosum62) cost matrix and gap penalties, while ribosomal sequences were added to the already existing alignments (option `-add`) using the MAFFT v. 7 online tool (Katoh et al., 2019) with default settings. Therefore, the final dataset comprised 2,838 species and 52 genes (43,605 nucleotide positions). We used the same partition scheme as in Zheng and Wiens (2016) and the GTRCAT model implemented in RAXML. To account for phylogenetic uncertainty in downstream analyses, RAXML was run 100 times on distinct starting trees under the default settings. A majority rule consensus tree (threshold 75%) was then inferred from the resulting 100 trees.

To estimate divergence times, we applied fossil calibration in MEGA-CC v. 11.0.10 (Tamura et al., 2021), which implements the RelTime method based on a relative rate framework (Tamura et al., 2012), thus providing an efficient approach for dating large phylogenies (Barba-Montoya et al., 2021). We used 11 fossil calibration points corresponding to calibration numbers 2, 4–7, 9–13 in Zheng and Wiens (2016) with the same age constraints as in Zheng and Wiens (2016) and Mulcahy et al. (2012). Computation of divergence times was performed using the RelTime-Branch Length algorithm (i.e., MEGA will not re-estimate branch lengths). To rule out an effect of outgroups on macro-evolutionary inferences, we excluded the outgroups prior to subsequent analyses.

We also assessed whether the results were consistent with inferences based on the lizard phylogeny by Tonini et al. (2016). This phylogeny includes additional taxa that may act as seed dispersers, but it is based on the supermatrix by Pyron et al. (2013), which only includes 17 genes. Using this supermatrix, Tonini et al. (2016) generated a distribution of 10,000 trees with a fixed topology for species with DNA-sequence data, and the remaining unsampled species were randomly assigned within their genus or higher-level clade. This phylogenetic tree may therefore not be suitable for inferences on character evolution rates (see the explanation in Tonini et al. 2016), and the main results presented here are thus based on the phylogenetic reconstruction described above. However, the main results between both phylogenies were qualitatively similar (see results).

2.3. Ancestral state reconstruction

In order to investigate the evolution of flower visitation and seed dispersal behaviour in Lacertilia (P1), we performed stochastic character mapping (Huelsenbeck and Crandall, 1997) using the R package *phytools* v. 1.2.0 (Revell, 2012). This method uses a Bayesian Markov-Chain-Monte-Carlo (MCMC) algorithm to sample character histories (i. e., presence or absence of seed dispersal or flower visitation) from their posterior probability distribution. We first used our best RAXML tree (i.e. with the highest overall log likelihood) to compare the fit of a model with equal forward and backward transition rates ('ER') to one with unequal transition rates ('ARD') using likelihood ratio tests. The ARD model (i.e., the rate of innovation of seed dispersal/flower visitation differs from the rate of loss of seed dispersal/flower visitation) fitted the data significantly better ($p < 0.05$) than the equal rate model. To account for phylogenetic uncertainty, we then computed a stochastic character map for 100 random trees from the posterior distribution of the RAXML analysis using the ARD model (function '*make.simmap*'). The 100 stochastic character maps were then summarised across all trees and

the posterior probabilities of trait states were visualised on the best RAXML phylogeny.

Furthermore, we conducted a sensitivity analysis to evaluate if our results were robust to missing data, i.e., species (and their mutualistic behaviour) not sampled in our phylogeny. These were performed in the R package *SensiPhy* v. 0.8.5 (Paterno et al., 2018). Specifically, we compared the percentage change in inferred transition rates from our full dataset to those obtained using the same model (ARD), but randomly deleting 10%, 20%, 30% or 40% of the species in our phylogeny. For each level of data completeness (10%, 20%, 30% or 40% of the species missing), we repeated the analysis 500 times, i.e., deleting information for a different set of species each time. Following previous studies (Miller and Stroud, 2022; Paterno et al., 2018), we considered a mean change of $\leq 10\%$ in the transition rate parameter estimates minimal, suggesting that missing data probably did not substantially affect our inferences.

2.4. Rate heterogeneity in ancestral state reconstructions

Heterogeneity in trait evolution rates may affect inferences of ancestral states, and may thus bias our results (Maddison, 2006). To evaluate this bias, we also inferred ancestral states using Hidden Markov models (HMM) that consider transition rate heterogeneity between lineages by assuming any number of unobserved ('hidden') rate categories that can account for the evolution of flower visitation or seed dispersal behaviour, and we compared these reconstructions to those obtained from the simple ARD model. To do so, we compared the fit of four models using the R package *corHMM* v. 2.8 and the function *corHMM* (Beaulieu et al., 2022): 1) a simple ER model, 2) a simple ARD model, 3) an ER model that accounts for rate heterogeneity by assuming two hidden rate categories (i.e., distinguishing between 'slow' versus 'fast' evolution), and 4) an ARD model that accounts for rate heterogeneity by assuming two hidden rate categories. The best model was selected based on the lowest Akaike information criterion corrected for small datasets (AICc). Ancestral state reconstructions using 500 stochastic character mappings were then repeated under the transition rates inferred for the best model, for each interaction type, and compared to those obtained when hidden rates were not considered (see before).

2.5. Correlated evolution between seed dispersal and flower visitation

To evaluate whether flower visitation (presence/absence) and seed dispersal (presence/absence) were evolutionarily correlated (P2), we used *BayesTraits* v. 3.0.1 (Meade and Pagel, 2016). We used the *discrete* function to compare a model of dependent (correlated) trait evolution (i. e., seed dispersal evolution depends on flower visitation evolution or vice versa) with one of independent (uncorrelated) trait evolution (i.e., seed dispersal and flower visitation evolve independently). To account for phylogenetic uncertainty, we included all 100 RAXML trees in the analysis. We used a reversible-jump MCMC algorithm, which simultaneously explores model and parameter space without the need to define a model a-priori, and avoids over-parameterisation. A hyperprior, with the mean drawn from a uniform distribution between 0 and 10, was used in order to reduce the arbitrariness of setting informative priors (Pagel and Meade, 2006). To facilitate parameter estimation and avoid very small estimates, the branches of the phylogenetic trees were scaled to a mean length of 0.1 (Meade and Pagel, 2016). For each analysis, we performed five runs for 10,000,000 generations, sampling every 1,000 generations and using a burn-in of 10%. MCMC diagnostics were checked for convergence in *Tracer* v. 1.7.1 (Rambaut et al., 2018). Support for the dependent trait evolution model over the independent model was assessed by comparing their marginal likelihoods by calculating the log Bayes Factor (BF), where a BF > 2 indicates correlated evolution of the two traits analysed. We estimated the marginal likelihood of each model using a stepping stone sampler with 100 stones and

100,000 iterations for each stone.

Moreover, since the model for correlated (dependent) trait evolution was strongly favoured over the uncorrelated (independent) model, we also evaluated if the transition rate of evolving flower visitation was conditional on the presence of seed dispersal, and *vice versa*. Hence, we compared BF_s of the full unconstrained model of dependent trait evolution (see before) to a model where the transition rate from non-flower-visiting to flower-visiting was constrained to be equal for lineages with or without seed dispersal, and a model where the transition rate from non-seed-dispersal to seed-dispersal behaviour was constrained to be equal for lineages with or without flower visitation. MCMC and model settings followed those described above.

To corroborate the *BayesTraits* findings, we also evaluated the evolutionary association between the presence/absence of seed dispersal and flower visitation behaviours across Lacertilia taxa using a phylogenetic logistic regression (PLR) approach for binary traits, which is a regression analysis that accounts for phylogenetic non-independence of data points (i.e., species). Prior to PLR, the set of 100 trees was rescaled to a minimum edge length of 0.00001 using the R package *paleotree* v. 3.4.5 (Bapst, 2012) to avoid problems with zero branch lengths. The response variable was the presence/absence of seed dispersal behaviour, and the explanatory variable flower visitation, although a model in which these were exchanged yielded identical results. PLR was carried out using the package *phylolm* v. 2.6.2 (Tung Ho and Ané, 2014) with the function *phylolm* and the method *logistic.MPLE* that aims to maximize the penalized likelihood. Furthermore, we performed a sensitivity analysis to evaluate the robustness of our results to missing data, i.e., assessing model estimates when removing up to 40% of the species data, using the same approach as outlined above for the ancestral state reconstructions.

2.6. Diversification rates

We also assessed whether Lacertilia lineages with seed dispersal or flower visitation behaviours had higher diversification rates than lineages lacking these behaviours (P3). Therefore, we fitted character-dependent (CD) and character-independent (CID) diversification rate models that may take 'hidden' states into account to our phylogenetic data (Beaulieu and O'Meara, 2016). In our character-independent models, net turnover rates (i.e., $\lambda + \mu$, in which ' λ ' refers to speciation rate and ' μ ' to extinction rate), as an approximation of diversification, were unlinked from observed state combinations, whereas in the character-dependent models, rates of turnover were allowed to vary across the different states. For all models (CD and CID) we fixed the extinction fraction (' ϵ ', calculated as μ / λ) to be the same for each trait and hidden state (if present), and accounted for missing taxa by estimating the sampling fraction for each trait based on the percentage of flower visitors and seed disperser inferred in our dataset. Specifically, approximately 4,480 currently described lizard species are missing from our phylogenetic data, and none of these have been recorded as flower visitor or seed disperser (Uetz et al., 2022). We fitted four models to our data using the *hisse* function in the R package *hisse* v. 2.1.10 (Beaulieu and O'Meara, 2016): 1) a 'null' model in which turnover rates were the same for both states (e.g., presence or absence of seed dispersal), no hidden states were considered but transition rates varied according to the ARD model ('CID' model), 2) a character-dependent model in which turnover rates were unlinked between the states, i.e. state-dependent diversification, and no hidden states were considered ('CD' model), 3) a character-independent hidden state model in which turnover rates were the same for both states, but were allowed to differ for two hidden states ('CID2' model) and 4) a character-dependent hidden state model in which turnover rates were unlinked between the states and were also allowed to differ for two hidden states ('CD2' model). For model comparisons, we used the AICc. Support for a character-dependent ('CD') model would suggest that our mutualistic trait may have influenced diversification rate variation across Lacertilia lineages. Hereafter, tip-

associated net diversification rates were computed under the best model (CD2) using the *GetModelAveRates* function in *hisse*.

3. Results

3.1. Prevalence of flower visitation and seed dispersal behaviour in Lacertilia

We recorded 64 flower visiting Lacertilia species (2.3% of a total of 2,837 species investigated in this study) across nine families (28 genera, [Supplementary Fig. S1](#)), of which at least 41 species (1.4% of a total of 2,837 investigated species) are most likely acting as pollinators. Seed dispersal behaviour was recorded for 382 species (13.5%) in 26 families (113 genera), and 41 species (1.4%) were both seed dispersers and flower visitors, thus potential 'double mutualists'. The families Gekkonidae and Dactyloidae included the highest number of species (15 and 12 species, respectively; [Supplementary Fig. S1](#)) for which flower visitation has been described. Seed dispersal has also been frequently recorded in the families Gekkonidae and Dactyloidae (57 and 46, respectively) but it was most prevalent in the family Scincidae (69 species; [Supplementary Fig. S1](#)). Double mutualists were most frequently recorded in Dactyloidae, in the well-studied genus *Anolis* (11 species).

3.2. Ancestral state reconstructions and transition rate heterogeneity

For seed dispersal and flower visitation behaviour modelled on the best RAxML phylogeny by Zheng and Wiens (2016) or the Tonini et al. (2016) phylogeny, the ARD model that included hidden transition rate categories (hereafter referred to as 'ARD + 2 rates' model) had the lowest AICc (see [Supplementary Table S2 and S3](#) for model comparison). Ancestral state reconstruction of seed dispersal and flower visitation behaviour revealed that flower visitation behaviour has evolved independently in each of the nine lizard families and, apart from Liocephalidae, multiple origins of this behaviour within a family may also be common ([Fig. 1](#), [Supplementary Fig. S2](#)). The probability of the ancestor of all Lacertilia (Squamata) to have been a flower visitor was low (posterior probability of ca. 0.3–0.4 for the ARD model and > 0.01 for the ARD + 2 rates model) and the first strongly supported occurrence (>0.8 posterior probability for both rate categories combined and averaged across all simulations) of flower visitation is maximum 50 million years old (Ma) ([Fig. 1](#), [Supplementary Fig. S2](#)). Nevertheless, in some of the 500 simulations, the ARD + 2 rate model suggested an earlier origin of flower visitation, but the combined posterior probability for both rate categories and averaged across all simulations was < 0.8 for those lineages.

For seed dispersal, inferences indicated uncertainty in the precise timing of the origin of this behaviour. While the ARD model based on 100 RAxML trees suggested that seed dispersal behaviour was probably already present in the ancestor (crown node) of all Lacertilia (ca. 160 Ma), and thereafter lost and regained in several lineages independently ([Supplementary Fig. S3](#)), the ARD + 2 rates model indicated a younger origin of seed dispersal behaviour, around 60–70 Ma ([Fig. 1](#)). Families belonging to the clade Pleurodonta (e.g., Liocephalidae, Iguanidae, Dactyloidae) likely shared a common origin of seed dispersal behaviour, while other families such as Gekkonidae, Phyllodactyloidae, Agamidae or Scincidae secondarily re-gained seed dispersal, independently from each other. Reconstructions on the phylogeny by Tonini et al. (2016) supported similar results as those obtained with the modified Zheng & Wiens phylogeny, with several independent origins of both mutualisms, and a later origin of flower visitation (ca. 50 Ma, [Supplementary Fig. S4, S6](#)) than seed dispersal behaviour (ca. 60–70 Ma, [Supplementary Fig. S5](#)), thus consistent with P1.

Our sensitivity analysis showed that even when up to 40% of the species are removed from the phylogeny, the mean expected change for the gain or loss of seed dispersal behaviour in Lacertilia under the ARD

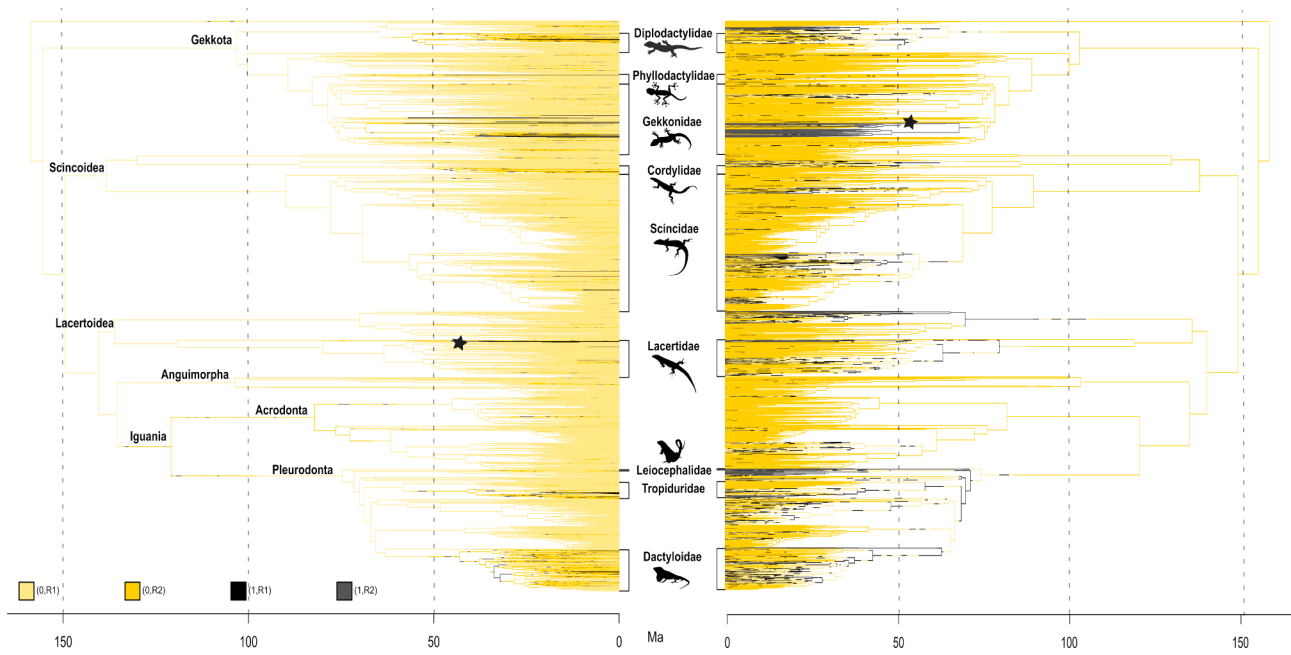


Fig. 1. Ancestral state reconstruction of flower visitation (left) and seed dispersal (right) behaviour in Lacertilia inferred using stochastic character mapping under the unequal transition rate model (ARD) including rate heterogeneity (+2 rates model) onto the best *RAXML* phylogenetic tree. One random mapping based on 500 simulations is shown. The posterior probability of ancestral nodes and branches to be flower visitor/seed disperser (black, '1') or not (yellow, '0') is indicated with the primary colours, whereas transition rate heterogeneity ('R1' and 'R2') is depicted in slight modifications in the intensity of those colours (light/dark). The first strongly supported origin (combined posterior probability > 0.8 for R1 and R2 combined and averaged across all simulations) of each behaviour is indicated with an asterisk. All Lacertilia families in which flower visitation has been recorded, and that also frequently include seed dispersal (i.e., families that contain potential double mutualistic lineages) are indicated at the tips. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

model was < 10% (see Supplementary Table S5, Supplementary Fig. S6). The mean change for the gain or loss of flower visitation behaviour was > 10%, but only when >20% of the species were removed (see Supplementary Table S5, Supplementary Fig. S7). However, even when up to 40% of the species were removed, the mean percentual change remained < 20%, suggesting only a moderate change in transition rate estimates due to missing data.

3.3. Correlated evolution of flower visitation and seed dispersal behaviour in Lacertilia

BayesTraits analyses strongly favoured a model of dependent

(average log marginal likelihood = -1131.41) over independent (average log marginal likelihood = -1173.84, BF > 10) trait evolution for seed dispersal and flower visitation behaviours (see Table S6 for individual run results). Moreover, the model of dependent (unconstrained) trait evolution suggested that the transition rate to flower visitation from seed-dispersing lineages (median rate = 0.312) was much higher than from non-seed dispersing lineages (median rate = 0.042). In addition, the model in which the transition to flower visitation was constrained to be the same for lineages with or without seed dispersal (average log marginal likelihood = -1141) performed worse than the full unconstrained model (average log marginal likelihood = -1131.41, BF > 10, Supplementary Table S6). However, the model in

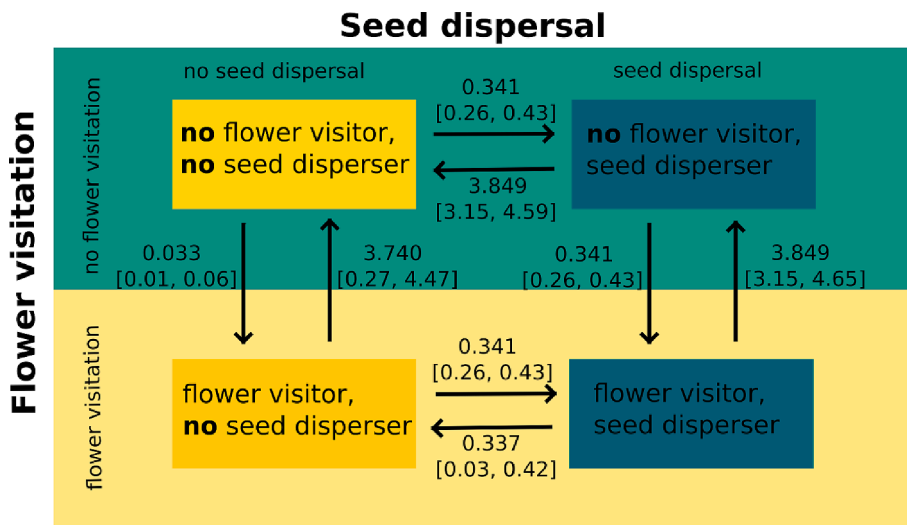


Fig. 2. Median evolutionary transition rates for seed dispersal and flower visitation behaviour in Lacertilia as estimated by five Markov Chain Monte Carlo runs in *BayesTraits* for the model of trait evolution with the highest average marginal likelihood and fewest number of parameters. This model illustrates how evolutionary transition rates between seed dispersal and flower visitation are correlated and unconstrained, except for the transition rates to seed dispersal behaviour, which were not significantly different for flower visiting and non-flower visiting lineages. In contrast, transition rates to flower visitation were much higher for seed-dispersing lineages compared to non-seed-dispersing lineages. 95% Highest Posterior Density Intervals (HPDI) for transition rate estimates are indicated in brackets.

which the transition to seed dispersal was constrained to be the same for lineages with or without flower visitation (average log marginal likelihood = -1131.84) performed slightly better than the full unconstrained model (BF = 0.856; Supplementary Table S6). Inferred transition rates under this best model are depicted in Fig. 2. Overall, these analyses suggest that the presence of seed dispersal may have pre-disposed lineages to evolve flower visitation and thus become double mutualists, but not necessarily *vice versa*, i.e. flower visitation did not favour the evolution of seed dispersal behaviour. The two independent runs for the dependent *versus* independent model of trait evolution using the Tonini et al. (2016) phylogeny also strongly favoured a model of correlated evolution (average log marginal likelihood = -1336.6) over independent evolution (average log marginal likelihood = -1384.11, BF > 10).

Finally, phylogenetic logistic regression also revealed a significant association between seed dispersal and flower visitation for all 100 trees analysed ($p < 0.001$). Even when up to 40% of the species were removed from the dataset, the mean change in the β -estimate was low (<10%, Supplementary Table S7, Supplementary Fig. S8, S9). Regardless whether seed dispersal or flower visitation was used as predictor variable, the β -estimate remained significant in all (100%) of the iterations (500 random deletion steps) (Supplementary Table S7, Supplementary Fig. S8, S9). These results support the prediction (P2) that seed dispersal activity and flower visitation are evolutionary linked, and that missing data probably do not affect this inference.

3.4. Diversification rates of flower visiting and seed dispersing lineages

Diversification rate analyses suggested that a model that assumed state-dependent diversification and two rate heterogeneity categories (CD2) provided the best fit to the flower visitation and seed dispersal data (Supplementary Table S4). Independent from the hidden state categories ('A' and 'B'), lineages acting as flower visitors had higher turnover rates – suggesting higher diversification rates – than lineages for which flower visitation was absent or not reported (net turnover rate for flower visitors, hidden state A = 0.0668 and B = 0.2786; for non-flower visitors, hidden state A = 0.0014 and B = 0.0709). The same applied to seed dispersal activity, where lineages acting as seed dispersers had higher turnover rates (net turnover rate for seed dispersers, hidden state A = 0.0674 and B = 0.2256) than lineages for which seed dispersal behaviour was absent or not reported (net turnover rate for non-seed dispersers, hidden state A = 2.061⁻⁹ and B = 0.0597). Parameter estimates of the best models are provided in Supplementary Table S8. Overall, these results suggest that the evolution of mutualistic behaviours in Lacertilia may have increased diversification rates via hidden character states, thus consistent with P3.

4. Discussion

We investigated the macroevolution of mutualistic interactions between lizards and plants to evaluate the evolutionary conditions under which double mutualists may evolve, using a novel dataset of flower visitation and potential pollination and seed dispersal behaviour by lizards (Lacertilia). Our models support our predictions of repeated evolutionary innovation of mutualisms (flower visitation, seed dispersal) across Lacertilia, the evolution of seed dispersal activity pre-dating flower visitation/pollination, strong evidence for correlated evolution between seed dispersal and flower visitation behaviours, and increased diversification rates on the evolution of either mutualistic behaviour. Furthermore, we show that the evolution of flower visitation occurs with transition rates > 10 times higher when seed dispersal activity is already present than in absence of seed dispersal activity, suggesting that double mutualisms may be evolutionary 'easier' to evolve than flower visitation and pollination behaviour by itself. This illustrates how seed dispersal behaviour may have predisposed Lacertilia lineages to evolve into double mutualists. Nevertheless, due to the sensitivity of double mutualisms to extinctions and perturbations, they may be

evolutionary labile, and not always persist over macroevolutionary time scales.

4.1. Data limitation

Although evidence for flower visitation and potential pollination was only recorded in ~ 2% of the investigated Lacertilia species, this may be an underestimate due to a lack of data, rather than true absence of this behaviour across Lacertilia lineages. Only recently, researchers became aware of a potential role of Lacertilia as flower visitors and pollinators, and there are only a few studies that systematically assessed this behaviour (but see Hansen and Müller 2009; García and Vasconcelos 2017; Hervías-Parejo et al. 2020; Correcher et al., 2023). Our study included slightly more Lacertilia species that may act as pollinators than Correcher et al. (2023; 41 *versus* 23 Lacertilia species). This may be because Correcher et al. (2023) only considered pollination behaviour for species for which pollination effectiveness was experimentally proven. Hence, our count of species acting as potential 'double mutualists' is also higher than that of Correcher et al. (2023; 41 *versus* 25 species). Our ancestral trait reconstructions revealed that flower visitation evolved independently in at least nine Lacertilia lineages, suggesting that flower visitation and pollination may occur more frequently in Lacertilia than was previously known. However, without more data, it is difficult to provide a precise estimate of the number of independent origins of this behaviour, and we may have overestimated the number of independent origins. Regarding frugivory and seed dispersal in Lacertilia, considerably more data were available compared to flower visitation. Still, as Valido and Olesen (2019) pointed out, for most Lacertilia, diet data are lacking, and thus it is likely that fruit-eating and seed dispersal by Lacertilia is also more common than currently documented. Our counts of frugivorous/seed-dispersing Lacertilia (382 in total) differed slightly from Valido and Olesen (2019), because not all the species they listed as frugivores (470 in total) had sequence data available, and could therefore be included in our phylogenetic analyses. Despite the current lack of data on both mutualistic interaction types, our sensitivity analyses revealed that our findings are robust to missing data, because even when up to 40% of the species were removed, the estimated transition rates to evolve (or lose) mutualistic behaviours remained very similar to inferences with the complete data. Nevertheless, we acknowledge that the sensitivity analyses do not completely cover the issue of distinguishing an actual absence of mutualistic lizard-plant-interactions (true negative) from a simple lack of records of such interactions or wrong trait state assignment (false negative; see e.g. Alves et al., 2017). Assessing how increasing proportions of incorrect trait state assignments impact transition rates, diversification rates, and hence ancestral state reconstructions will be a useful future direction, especially if interaction data remain limited. Interestingly, our age estimates for the ancestor of all Lacertilia (Squamata) and other major Lacertilia taxa (e.g. Gekkota, Scincoidea, Lacertoidea) were ca. 40–50 Ma younger than in Zheng and Wiens (2016; Supplementary Table S9). These different age estimates between our study and the Zheng and Wiens (2016) study may have resulted from using fewer fossil calibration points (due to the exclusion of taxa; see methods) or from using different approaches to estimate divergence times. Recent fossil evidence suggests an even earlier origin of modern Squamata at around 230 Ma (Whiteside et al., 2022). Hence, the origins of seed dispersal behaviour and flower visitation may be even older than we estimated, but relative estimates (i.e., seed dispersal preceding flower visitation) are unlikely to be affected.

To summarize, although our data have a few shortcomings, we are confident that our main conclusions (i.e. multiple *versus* a single origin of flower visitation and seed dispersal activity in Lacertilia and an earlier origin of the latter) are robust, because these findings hold even when using different phylogenies, and when accounting for phylogenetic uncertainty and missing data. Moreover, we believe that our findings will be further substantiated when more evidence for lizard-plant-

interactions becomes available. [Correcher et al. \(2023\)](#) inferred that the number of Lacertilia species interacting with flowers has increased fourfold since the last review on pollination service provided by Lacertilia ([Godínez-Álvarez, 2004](#)) (sevenfold compared to our count of pollinator species), and we are optimistic that future research will reveal even more mutualistic lizard-plant-interactions.

4.2. Evolution of flower visitation, pollination, and seed dispersal activity and interaction-relevant traits in Lacertilia

Flower visitation has been most frequently documented in the scientifically popular families Gekkonidae and Dactyloidae, which may be due to their high species numbers that increase the chance to observe the behaviour. However, the observed number of flower visitors within these two taxa is significantly different from random expectations ($\chi^2 = 8.819$, $p = 0.003$ for Gekkonidae and $\chi^2 = 12.176$, $p < 0.001$ for Dactyloidae), suggesting that species richness alone cannot account for this observation. Instead, the possession of specific traits in species belonging to Gekkonidae and Dactyloidae, such as traits relating to climbing abilities (to access trees/flowers) and/or colour vision (to detect flowers and coloured nectar), may facilitate the exploitation of floral resources. Indeed, species within Gekkonidae have relatively small body sizes, which may facilitate climbing and thereby accessing nectar or fruits of fragile plants compared to larger-bodied lizards ([Correcher et al., 2023](#)).

Interestingly, traits related to mutualistic interactions with plants may be phylogenetically conserved, consistent with a phylogenetic signal for flower visitation and seed dispersal (see [Supplementary Materials & Results](#)). For example, colour perception may facilitate the detection of fruits and seeds, and thus also influence the evolution of seed dispersal behaviour in Lacertilia, as exemplified in New Zealand ([Lord and Marshall, 2001](#)). [Lord and Marshall \(2001\)](#) showed that in 246 fleshy-fruited plant species indigenous to New Zealand, white and blue coloured flowers were significantly correlated with shrub and divaricate growth forms, small fruit size and open habitats, i.e., plant attributes linked to seed dispersal by small-bodied, open habitat lizards. This suggests that both flower visitation and seed dispersal behaviours evolve non-randomly across Lacertilia, which is in agreement with our study (also see [Supplementary Methods & Results](#)) and [Valido and Olesen \(2019\)](#). Additional traits related to the exploitation and sensory perception (e.g., olfaction) of flowers or fruits may further explain the evolution of flower visitation and seed dispersal in Lacertilia, similarly as it has affected the evolution of mutualistic behaviours in other vertebrates, e.g. ([Omer et al., 2018](#); [Onstein et al., 2020](#)).

4.3. Origins of Lacertilia seed dispersal and flower visitation

Our analyses suggest that seed dispersal behaviour pre-dated flower visitation in Lacertilia. Furthermore, although the temporal origin of seed dispersal activity varied according to the model we used, the model accounting for transition rate heterogeneity predicted a relatively young origin (ca. 60–70 Ma). Interestingly, this is consistent with the explosive radiation of angiosperms ([Ramírez-Barahona et al., 2020](#)), the evolution of fleshy fruits and biotic dispersal adaptations, and the contemporaneous diversification of most modern vertebrate groups ([Benton et al., 2022](#)), including frugivorous birds and mammals ([Tiffney, 2004](#)). Frugivorous birds, mammals and lizards thus possibly replaced earlier seed dispersal interactions with herbivorous dinosaurs ([Onstein et al., 2022](#)). The later origin of flower visitation in lizards (ca. 40–50 Ma) thus followed the hypothesised co-diversification of angiosperms and their insect pollinators after the Cretaceous-Paleogene mass extinction event ([Asar et al., 2022](#)).

Nevertheless, we suggest that an earlier origin of seed dispersal behaviour is possible as well. For example, some of the outgroups such as crocodiles (e.g., *Alligator mississippiensis*) or the tuatara (*Sphenodon punctatus*) – which is the only extant species in the order

Rhynchocephalia and sister group to Squamata – are known seed dispersers ([Platt et al., 2013](#); [Valido and Olesen, 2019](#)), but not flower visitors/pollinators. The same applies to other reptiles such as tortoises and turtles, which may act as seed dispersers ([Falcón et al., 2020](#)), but so far have not been observed to use floral rewards and thereby pollinate plants. Similarly, the oldest fossil evidence for herbivorous (possibly frugivorous) Lacertilia comes from the fossil *Kuwajimalla kagaensis* in Japan from the early Cretaceous (ca. 132–135 Ma, [Evans and Manabe 2008](#)). Thus, although flower visitation behaviour may be younger than 50 Ma, seed dispersal was maybe already present at ca. 160 Ma in the late Jurassic/early Cretaceous. However, giving a precise point estimate for the origin of each behaviour is difficult without more interaction and fossil data, and we therefore suggest time frames rather than time points in which flower visitation (ca. 50–40 Ma) and seed dispersal behaviour (ca. 230–60 Ma) have evolved within Lacertilia.

4.4. Correlated evolution between flower visitation and seed dispersal and double mutualisms

We also detected a significant correlation between the occurrence of flower visitation and seed dispersal behaviour in Lacertilia. Interestingly, most (41 out of 64) flower visiting Lacertilia species also act as seed dispersers, but there are still some Lacertilia taxa (e.g. *Bavayia robusta*, *Hemicordylus capensis*, *Haemodracon riebeckii*, *Hemidactylus* sp.) that act as flower visitor but not seed disperser or vice versa (e.g. Agamidae, Gerrhosauridae, Iguanidae). Moreover, our inferences of ancestral states and correlated evolution imply that frugivory may have pre-disposed Lacertilia to visit flowers, leading to the evolution of double mutualisms. This result may be biased by seed dispersing studies that coincidentally also recorded flower visitation behaviour, but we did not find this to be the case frequently.

The evolution of double mutualisms may also relate to the eco-evolutionary ‘arena’ where these interactions take place. For example, the occurrence of double mutualisms is positively related to rainfall, which directly affects flowering and fruit formation in plants, but also to resource-poor habitats, such as in desert and xeric habitats on the mainland ([Correcher et al. 2023](#)). However, both interaction types occur more frequently on islands than anywhere else, suggesting that double mutualism between Lacertilia and plants is also a typical island phenomenon (see [Correcher et al. 2023](#)), consistent with the occurrence of double mutualisms in other clades such as birds or mammals ([Fuster et al., 2019](#)). Indeed, island conditions may facilitate the evolutionary persistence of double mutualisms across macroevolutionary timescales, because there are fewer food resources and interaction partners available for lizards than on the mainland ([Fuster et al., 2019](#)). Thus, high lizard densities due to a scarcity of predators and a lack of insect species (i.e., pollinators for the plants or food for the lizards) may be the island characteristics that have led to frequent flower and fruit visitation ([Olesen and Valido, 2003](#)) as well as the evolution and persistence of double mutualisms.

4.5. Evolutionary lability and diversification of mutualistic Lacertilia lineages

Ancestral trait reconstructions revealed a great evolutionary lability for seed dispersal and flower visitation behaviours ([Fig. 1](#), [Fig. S2](#), [S3](#)) and diversification rate inferences indicated that Lacertilia with mutualistic behaviours had higher diversification rates than lineages in which this behaviour was absent or not recorded. This suggests that Lacertilia lineages with mutualistic interactions can quickly evolve, but may be ‘ephemeral’, i.e., not always persisting over macroevolutionary times ([Rosenblum et al., 2012](#)). Indeed, the evolution of mutualistic behaviours may be associated with low ‘costs’ and high ‘gains’, especially in environments where food is scarce, but dependence on mutualistic interactions may increase vulnerability to extinction ([Fuster et al., 2019](#)). Although mutualistic interactions often seem to spur diversification in

angiosperms, for example due to interactions with pollinators (reviewed in van der Niet et al., 2014; van der Niet and Johnson, 2012) as shown in Andean bell flowers (Campanulaceae; Lagomarsino et al., 2016) or Neotropical *Ruellia* (Acanthaceae; Tripp and Tsai, 2017), or due to interactions with seed dispersers, as shown in tropical palms (Onstein et al., 2017, 2022) and plants that rely on seed-dispersing ants (Lengyel et al., 2009), mutualisms may also restrict diversification, as suggested by theoretical modelling (Yoder and Nuismer, 2010). This may be due to the constraints of ‘trait matching’ in mutualisms, which may allow only limited trait variation and therefore may counteract diversification (Yoder and Nuismer, 2010). Moreover, the mechanisms by which mutualisms influence diversification and how microevolutionary processes link to macroevolutionary patterns, require further investigation (Harmon et al., 2019; Hembry and Weber, 2020).

To conclude, our study provides the first insights into the macroevolution of flower visitation and seed dispersal behaviours by Lacertilia, indicating that Lacertilia seed dispersal behaviour is evolutionary older, and may have facilitated the evolution of flower visitation and thus double mutualisms. Although our study illustrates that double mutualisms in Lacertilia may evolve more frequently than previously thought, their prevalence on islands and xeric habitats suggests that their evolution may be evolutionarily labile, and their persistence conditional on extrinsic factors, such as resource availability (Olesen and Valido, 2003) or rainfall patterns (Correcher et al., 2023). Further studies and observational data on lizard-plant interactions are urgently needed to substantiate the findings of our analyses and test additional hypotheses on the occurrence and evolution of double mutualisms between lizards and plants. Specifically, identifying both the ecological conditions and the functional traits that favour (double) mutualisms between lizards and plants is essential to gain a better understanding of the ecology and evolution of these fascinating interactions, which are a fundamental and potentially threatened aspect of the unique ecological communities in which they are embedded.

CRediT authorship contribution statement

Belinda Kahnt: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Panagiotis Theodorou:** Methodology, Writing – review & editing. **Annegret Grimm-Seyfarth:** Resources, Writing – review & editing. **Renske E. Onstein:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

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

Data availability

All data used in this study can be found in the [supporting information](https://doi.org/10.6084/m9.figshare.23460260) or in figshare (<https://doi.org/10.6084/m9.figshare.23460260>).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2023.107839>.

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