Allometric relationships shape foreleg evolution of long-legged oil bees (Melittidae: *Rediviva*)

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Exaggerated traits of pollinators have fascinated biologists for centuries. To understand their evolution, and their role in coevolutionary relationships, an essential first step is to understand how traits scale allometrically with body size, which may reveal underlying developmental constraints. Few pollination studies have examined how traits can adaptively diverge despite allometric constraints. Here, we present a comparative study of narrow-sense static and evolutionary allometry on foreleg length and body size of oil-collecting bees. Concurrently, we assess the relationship between scaling parameters and spur lengths of oil-secreting host flowers. Across species and populations, we found low variation in static slopes (nearly all <1), possibly related to stabilizing selection, but the static intercept varied substantially generating an evolutionary allometry steeper than static allometry. Variation in static intercepts was explained by changes in body size (∼28% species; ∼68% populations) and spur length (remaining variance: ∼36% species; ∼94% populations). The intercept–spur length relationship on the arithmetic scale was positive but forelegs did not track spur length perfectly in a one-to-one relationship. Overall, our study provides new insights on how phenotypic evolution in the forelegs of oil-collecting bees is related to the variability of the allometric intercept and adaptation to host plants.

KEY WORDS: Exaggerated traits, plant–pollinator coevolution, static and evolutionary allometry, scaling parameters, constraint, adaptation.

How allometric relationships change within and between species is central to understanding phenotypic variation and the evolution of trait morphology (Cheverud 1982; Emlen and Nijhout 2000; Pélabon et al. 2014; Bright et al. 2016). Huxley's allometric model assumes that the growth rates of morphological traits are proportional to the growth of body size and follow a power function of the form,

$$Y = aX^b \tag{1}$$

where Y is trait size and X is body size (Huxley 1924, 1932). Given that growth is inherently a multiplicative process, allo-

metric relationships are often analyzed on a log–log scale where a power function yields a linear equation with intercept $\log(a)$ and slope b, which is reflective of allometry in its narrow-sense (Firmat et al. 2014; Pélabon et al. 2014, Pélabon et al. 2018). Three levels of allometry are recognized: *ontogenetic* (within individuals across developmental stages); *static* (across individuals at a similar developmental stage within a population); and *evolutionary* (across separate evolutionary lineages) (Gould 1966; Cheverud 1982; Pélabon et al. 2014). The differences among the three levels of allometry correspond to distinct types of variation

resulting from the growth of individuals, intraspecific variation, and phylogeny.

In insects, morphological traits typically show weak negative allometry, that is, $b < 1$ (Emlen and Nijhout 2000; Bondurianksy and Day 2003; Voje 2016). There are, however, exceptions, particularly traits under sexual selection, in which one or a few traits show positive allometry, that is, $b > 1$, and grow disproportionately or are exaggerated (Klingenberg and Zimmermann 1992; Emlen and Nijhout 2000; Shingleton et al. 2007; Lavine et al. 2015; but see Eberhard et al. 1998). Many studies on the allometry of exaggerated traits in insects have therefore been in the context of sexual selection (e.g., sexual signaling ornaments or weapons) (Emlen and Nijhout 2000). Much less is known about the allometry of exaggerated traits resulting from other types of selection, such as coevolution (Toju and Sota 2006). Plant-pollinator mutualisms have been shown to promote the coevolution of exaggerated traits (Darwin 1862; Nilsson 1988; Steiner and Whitehead 1990; Manning and Goldblatt 1997). These include the evolution in pollinating insects of extremely long mouthparts, as seen in some hawk moths (Darwin 1862; Nilsson 1988), certain butterflies (Kunte 2007; Bauder et al. 2015), euglossine bees (Borrell 2007; Düster et al. 2018), and some tabanid (Morita 2008) and nemestrinid (Manning and Goldblatt 1997) flies. These exaggerated traits are typically interpreted as local adaptations in response to differing selection gradients (e.g., from interactions with host plants), suggesting either a decoupling of variation in trait and body size (Steiner and Whitehead 1990; Johnson and Steiner 1997; Pauw et al. 2017) or high evolvability of the allometric parameters (Emlen and Nijhout 2000; Frankino et al. 2005; Voje and Hansen 2013; Pélabon et al. 2014; Voje et al. 2014).

Evidence that allometric relationships (static slope and intercept) can evolve and adapt, often rapidly, comes from two sources. First, experimental evidence from artificial selection on trait size demonstrates that it is possible for the allometric intercept (Frankino et al. 2007; Egset et al. 2012; but see Stillwell et al. 2016) and the slope (Bolstad et al. 2015) to evolve in response to targeted selection. Second, comparative studies analyzing the differences in the allometric relationships of closely related insect taxa provide additional evidence of their potential to undergo adaptive evolution (Emlen and Nijhout 2000; Frankino et al. 2005; Toju and Sota 2006; Shingleton and Frankino 2013; Pélabon et al. 2014). Of the two allometric parameters, the static slope has been shown to be less evolvable than the static intercept across species, with even less capacity to evolve across populations and recently diverged taxa (Voje et al. 2014). In contrast, the allometric-constraint hypothesis identifies allometry as a potential constraint on the evolution of morphological traits, on the basis that allometric relationships reflect strong functional, physiological, or other biological mechanisms that con-

strain the rate and direction of evolution (Huxley 1924; Gould 1966; Pélabon et al. 2014; Voje et al. 2014). Underlying the evolution of the scaling relationship are proximal developmental mechanisms that regulate and integrate trait growth (Emlen and Nijhout 2000; Shingleton and Frankino 2013). Only through selection on developmental mechanisms can changes in the scaling relationship and ultimately the trait evolve (Frankino et al. 2005; Emlen et al. 2007, Emlen et al. 2012; Shingleton and Frankino 2013). The adaptability of a trait is therefore potentially curtailed by these mechanisms and traits constrained along these narrow evolutionary trajectories appear less able to respond to changes in selective pressures (Voje and Hansen 2013; Pélabon et al. 2014; Voje 2016; Houle et al. 2019; Tsang et al. 2019). Furthermore, there is strong evidence that aspects of allometry, particularly the slope, evolve relatively slowly, on the scale of millions of years, and that their evolution is constrained at microevolutionary time scales (Pélabon et al. 2014; Voje et al. 2014; Bolstad et al. 2015; Houle et al. 2019).

The evolvability of allometry, or how it might shape phenotypic evolution of elongated or exaggerated traits in insect pollinators, has not been considered in sufficient detail. This includes its influence on pollinators in coevolutionary interactions. Thus, the constraint hypothesis seems in conflict with findings that exaggerated traits of pollinators are highly adaptable at the population level, that is, a decoupling of the variation in trait and body size (Steiner and Whitehead 1990; Johnson and Steiner 1997; Anderson and Johnson 2008; Pauw et al. 2017). Evidence supporting the constraint hypothesis also appears in conflict with models of coevolution, for example, arms race or pollinator shift models (Vogel 1984; Whittall and Hodges 2007; Johnson and Anderson 2010). Although the end-point of these interactions appear to have resulted in the elongation of interactive traits in several insect taxa, obtaining this adaptation requires selection at the developmental-genetic level and longer evolutionary time scales in order to follow this evolutionary trajectory (Bolstad et al. 2015; Houle et al. 2019).

Several pollination studies have investigated the role of body size as an alternative explanation to other evolutionary models as to why some insect pollinators have evolved exaggerated traits (see Johnson and Anderson 2010 for a review). The current consensus amongst these studies is that there appears to be a decoupling between the variation of the exaggerated trait and body size. However, these studies did not estimate static allometric relationships for species or populations and are largely based on allometry in the broad sense (Houle et al. 2011; Voje and Hansen 2013; Pélabon et al. 2014), incorporating mean body size as a covariate in regression models when assessing variation in mean trait size (e.g., Steiner and Whitehead 1990; Pauw et al. 2009, Pauw et al. 2017; Johnson and Anderson 2010). Consequently, this limits the ability to test if there are

developmental constraints on morphological trait evolution in these pollinators.

An interesting aspect of estimating narrow-sense parameters is that it allows one to assess if these parameters can evolve adaptively in relation to the strength of different types of selection and therefore how allometry may influence trait evolution (Houle et al. 2011; Voje et al. 2014; Higginson et al. 2015; Voje 2016). Directional selection can favor steeper allometric slopes (e.g., Voje 2016), whereas stabilizing selection leads to shallower slopes (e.g., Eberhard 2009; Pélabon et al. 2011). Different adaptive landscapes can also select on trait means (intercept) or trait variances that can influence the steepness of the slope in different ways (Pélabon et al. 2014). Within plant–pollinator coevolution, selection gradients can range from strongly directional (e.g., where exaggerated traits confer fitness advantages (Pauw et al. 2009; Karolyi et al. 2013)), to stabilizing selection where the accuracy of fit between interactive traits is required (e.g., Berg 1960; Pélabon et al. 2011; Armbruster and Wege 2019). Selection gradients also shift across the spatial structure of populations of interacting species and in relation to the diversity of the community of interacting species within these populations (Thompson 2005; Pauw et al. 2009).

When considering selection via an external agent (e.g., flower size), it is not always clear which scale provides the best biological meaning (Packard 2018; Pélabon et al. 2018). Under an adaptive scenario, the use of the log-log scale may not be justified anymore and the relationship between static allometric intercept of a pollinator and flower size may be better analyzed with both variables expressed on the arithmetic scale. If there is selection for a close match between linear pollinator and flower traits, one would expect a possible one-to-one relationship on an absolute scale: a 1 mm change in floral trait size should trigger a 1 mm response in a pollinator trait size (Nilsson 1998; Armbruster et al. 2009). Such a relationship is not easily interpreted on the log-log scale.

The long-legged oil-collecting bees (Hymenoptera: Melittidae) represent an excellent model system to explore the relative contribution of allometric adaption or constraint in the evolution of a pollinator trait. The center of diversity for this family is in South Africa, where the oil-collecting genus *Rediviva* displays a wide range in absolute and relative foreleg length across its 26 species. Species range from those with elongated forelegs (e.g., *R. emdeorum*) longer than the body to those with forelegs (e.g., *R. albifasciata)* shorter than the body (Table 1; Whitehead and Steiner 2001; Whitehead et al. 2008). Several studies invoke adaptation and coevolutionary changes in which changes in floral spur length select for changes in foreleg length mostly independent of body size (Steiner and Whitehead 1990, 1991; Pauw et al. 2017). However, some species appear to show foreleg variation correlated with body size variation (Steiner and Whitehead

1991). Pauw et al. (2017) show that evolutionary change in foreleg length and body size appears to be weakly correlated, suggesting that an increase in trait size is not linked to an increase in body size. Rather, the floral spur length of their plant hosts has been shown to be the main driver of foreleg length, seemingly independent of allometry (see also Johnson and Anderson 2010).

On account of the mixed results from these studies, and particularly a lack of consideration of allometric scaling parameters and how these change at the different levels of allometry (static and evolutionary), it is unclear which processes are potentially involved in the elongation of foreleg length. Certainly, allometry has been shown to be important in other traits (e.g., mouthparts) for *Rediviva* (Melin et al. 2019).

We present new morphological trait data, together with a comprehensive analysis on the allometric scaling relationships for *Rediviva* to assess to what extent allometry represents constraints on phenotypic evolution. We ask do allometric relationships generated by the correlated development of the forelegs and the body constrain the evolution of foreleg length in oil-collecting bees, or does variation in foreleg length among species and populations reflect adaptation to host plants with different spur lengths. We predict that if the developmental constraints that generate static allometry are important in shaping evolutionary allometry (i.e., constraint hypothesis), we expect static allometric parameters to be similar among species and populations because they have low evolvability. In an extreme case, we also expect evolutionary allometry to be similar to static allometry if both the allometric intercept and the slope do not evolve among species and populations. If the low evolvability of the allometric parameters constrains the evolution of foreleg length across species and populations, we also expect a weak or no relationship between spur length of the host plants and allometric parameters. In contrast, if foreleg length is mostly shaped by selection (i.e., adaptive hypothesis) then allometric parameters may be correlated with the length of the spurs of their host plants. Our results provide new insights into how pollinator traits change under a selective pressure within a plant-pollinator mutualism and contribute to a broader understanding of these coevolutionary systems.

Materials and Methods
OIL-BEE DATA COLLECTION AND MORPHOLOGICAL MEASUREMENTS

We measured the foreleg lengths (Whitehead and Steiner 2001) and inter-tegula distance (referred to as body size from here on) (Melin et al. 2019) of the female bees for 25 of the 26 known species of *Rediviva* ($n = 7$–147; Table 1) from a range of localities within their known distribution, where feasible. We also measured the foreleg and body size for 10 populations of the highly variable species *R. neliana* ($n = 7$–20, Table 2). All

Table 1. Summary of morphological data and allometric parameters for 25 species of *Rediviva* bees. LegLength: mean total length of foreleg length [mm]; LL.SD: standard deviation of leg length; BodySize: inter-tegula distance as a measure of body size; BS.SD: standard deviation of body size; intercepts: parameter *a* of the allometric relationship; slopes: parameter *b* of the allometric relationships; lcl.i, ucl.i: lower and upper 95% confidence limits of parameter *a*; lcl.s, ucl.s: lower and upper 95% confidence limits of parameter *b*.

Taxa	N	LegLength	LL.SD	BodySize	BS.SD	intercepts	slopes	lcl.i	ucl.i	lcl.s	ucl.s
Rediviva albifasciata	29	5.86	0.17	2.51	0.15	4.60	0.26	3.31	6.38	−0.09	0.62
Rediviva alonsoae	11	8.10	0.38	3.33	0.16	4.93	0.41	2.06	11.77	−0.31	1.14
Rediviva aurata	30	9.07	0.51	2.75	0.14	4.08	0.79	2.71	6.15	0.38	1.19
Rediviva autumnalis	19	13.92	0.43	3.17	0.14	10.34	0.26	5.32	20.11	−0.32	0.83
Rediviva brunnea	15	11.78	0.54	3.18	0.11	9.27	0.21	3.52	24.39	−0.63	1.04
Rediviva colorata	39	10.49	0.65	3.00	0.24	9.29	0.11	7.06	12.25	−0.14	0.36
Rediviva emdeorum	45	20.14	1.39	3.36	0.16	8.76	0.69	5.61	13.70	0.32	1.05
Rediviva gigas	8	10.82	0.47	4.20	0.10	3.43	0.80	0.29	40.23	−0.92	2.52
Rediviva intermedia	19	9.10	0.28	3.26	0.16	5.16	0.48	2.75	9.69	−0.05	1.01
Rediviva intermixta	18	8.05	0.27	2.96	0.17	5.58	0.34	3.33	9.36	−0.14	0.81
Rediviva longimanus	39	17.43	0.91	3.19	0.19	8.91	0.58	6.29	12.63	0.28	0.88
Rediviva macgregori	35	12.86	0.64	3.19	0.16	7.05	0.52	4.58	10.84	0.15	0.89
Rediviva micheneri	41	13.79	0.63	2.84	0.15	7.05	0.64	5.02	9.90	0.32	0.97
Rediviva neliana	147	8.79	1.74	2.46	0.37	3.35	1.06	3.16	3.55	1.00	1.13
Rediviva nitida	31	9.29	0.76	3.02	0.19	3.56	0.87	2.51	5.04	0.55	1.18
Rediviva pallidula	64	10.20	0.53	2.94	0.17	6.54	0.41	5.05	8.48	0.17	0.65
Rediviva parva	50	7.43	0.52	2.85	0.18	3.26	0.78	2.50	4.27	0.53	1.04
Rediviva peringueyi	19	9.74	0.39	3.09	0.17	6.90	0.31	4.01	11.86	−0.17	0.79
Rediviva rhodosoma	24	12.44	0.47	3.08	0.17	6.34	0.60	3.90	10.30	0.17	1.03
Rediviva ruficornis	30	9.54	0.36	2.78	0.09	5.95	0.46	3.18	11.15	−0.15	1.07
Rediviva rufipes	49	7.47	0.32	2.93	0.14	5.33	0.31	3.68	7.73	−0.03	0.66
Rediviva rufocincta	66	8.05	0.52	2.75	0.14	3.56	0.80	2.66	4.77	0.52	1.09
Rediviva saetigera	23	7.09	0.27	2.94	0.13	4.37	0.45	2.40	7.94	−0.11	1.00
Rediviva transkeiana	7	10.01	0.06	2.74	0.10	9.20	0.08	2.78	30.43	−1.10	1.27
Rediviva whiteheadi	11	10.88	0.22	3.15	0.09	11.48	−0.05	2.60	50.67	−1.34	1.25

measured specimens were from the Iziko Museums of South Africa and had a foreleg removed from the bee and mounted on a card (Steiner and Whitehead 1990, 1991) that made it easy to measure leg length. Following Steiner and Whitehead (1990, 1991; Figure 1), we took individual measurements of the segments: tarsus (disti-, medio-, and basitarsus), tibia, femur, and trochanter to estimate total foreleg length. Unlike Steiner and Whitehead (1990, 1991), we excluded the coxa from total foreleg length as we felt this introduced additional error owing to the coxa being damaged when the leg was removed from the body. Both traits were measured using a Leica Z16 APO stereoscope (Leica Application Suite software; version 4.7.1, 2003–2015).

OIL HOST PLANT TRAIT DATA

Rediviva bees have been recorded to collect floral oil from 14 plant genera within four families Iridaceae, Orchidaceae, Scrophulariaceae and Stilbaceae. Using plant visitation records available from museum records and publications (Whitehead and Steiner 2001; Whitehead et al. 2008; Pauw et al. 2017; Iziko Museums of South Africa 2018), we generated a list of oil host

plants for each *Rediviva* species, combined this with published spur length data (Snijman and Steiner 2013; Johnson and Bytebier 2015; Pauw et al. 2017; see Table S2 and references therein) and calculated a mean spur length across recorded host plants for each bee species.

In our choice of using mean spur length to characterize the selection from their host plants, we follow a recent evolutionary study concerning oil bees (Pauw et al. 2017). Since our bee species trait data are based on specimens collected from a wide range of localities, we think that mean spur length best represents host plant use across a bee species' range. It would be interesting to examine the variation in spur length available to individual bees within and across populations but obtaining such detailed data was not feasible across the number of species and the geographic range of our study.

DATA ANALYSIS

Full details of the methods including code needed to reproduce all analyses are provided as Supporting Information (all data used for the analysis may be obtained from the University of Cape

Table 2. Summary of morphological data and allometric parameters for 10 populations of *R. neliana*. LegLength: mean total length of foreleg length [mm]; LL.SD: standard deviation of leg length; BodySize: inter-tegula distance as a measure of body size; BS.SD: standard deviation of body size; intercepts: parameter *a* of the allometric relationship; slopes: parameter *b* of the allometric relationships; lcl.i, ucl.i: lower and upper 95% confidence limits of parameter *a*; lcl.s, ucl.s: lower and upper 95% confidence limits of parameter *b*.

Population	N	LegLength	LL.SD	BodySize	BS.SD	intercepts	slopes	lcl.i	ucl.i	lcl.s	ucl.s
R. neliana_1	20	8.54	0.42	2.69	0.15	4.74	0.60	3.72	6.04	0.35	0.84
R. neliana_11	15	8.47	0.66	2.21	0.19	4.51	0.79	3.86	5.27	0.60	0.99
R. neliana_18	15	9.17	0.97	2.52	0.28	3.98	0.90	3.46	4.58	0.75	1.05
R. neliana_2	15	7.48	1.09	2.35	0.34	3.25	0.98	2.96	3.56	0.87	1.09
R. neliana_21	15	13.00	0.50	3.20	0.14	6.94	0.54	4.61	10.46	0.19	0.89
R. neliana_22	7	10.15	0.29	2.48	0.11	6.59	0.48	3.96	10.97	−0.08	1.04
R. neliana_3	15	7.56	0.84	2.34	0.34	4.02	0.74	3.67	4.40	0.64	0.85
R. neliana_4	15	7.27	0.30	2.12	0.10	4.26	0.71	3.30	5.50	0.37	1.05
R. neliana_6	15	8.10	0.22	2.35	0.08	6.37	0.28	4.36	9.30	−0.16	0.73
R. neliana_8	15	9.01	0.99	2.31	0.27	4.23	0.90	3.75	4.78	0.76	1.05

Figure 1. Photographs of the lateral (A) and dorsal (B) view of *Rediviva longimanus* ♀. Morphological traits are depicted by the following letters: a = disti + mediotarsus; b = basitarsus; c = tibia; d = femur; e = trochanter; f = coxa and g = inter-tergula distance (body size). All traits were measured with the exception of f owing to the coxa typically being damaged when the leg was removed from the body. To calculate the total foreleg length, we added traits a to e.

Town's data portal; DOI: 10.25375/uct.13297640). Here, we give a brief overview of the species-level data analyses, which we carried out using the program R version 4.0.0 (R Core Team 2020). We used an equivalent analysis of our population-level data for *R. neliana* (SI §8).

Estimating the static allometric parameters

To estimate the static allometric relationships for each *Rediviva* species, we fitted a linear regression model with log(foreleg length) as a response variable and log(body size) as an explana-

tory variable. We added species and the interaction between species and log(body size) to the model to obtain direct maximum likelihood estimates of the two parameters of the allometric relationship for each species. We estimated the between-species variance in allometric slopes, corrected for sampling variance, as the variance of the estimated species-specific slopes minus the mean of the squared standard errors of the species-specific slopes (Hansen and Bartoszek 2012).

Comparison of static and evolutionary allometric relationships of Rediviva species

To compare the static and evolutionary allometric relationships, we used multilevel models (van de Pol and Wright 2009); also known as contextual models (Heisler and Damuth 1987; Egset et al. 2011) fitted using the "lme" function in the R package *nlme* (Pinheiro et al. 2017). The key model was:

$$l_{ij} = (\beta_0 + \mu_{0j}) + (\beta_\omega + \mu_{\omega j})(b_{ij} - b_{\cdot j}) + \beta_b b_{\cdot j} + \epsilon_{ij} \quad (2)$$

where l_{ij} is the logarithm of the foreleg length measurement of the ith individual of species j, b_{ij} is the logarithm of the body size of that individual, $b_{\cdot j}$ is the logarithm of the mean body size across all individuals of species j, β_0 is the intercept, that is, log(a) of the allometric relationship in Equation 1, β_w is the within-species effect, that is, the static allometric slope, β_b is the between-species effect, that is, the evolutionary allometric slope, and ϵ_{ij} are the residuals. The residuals are assumed to follow a normal distribution with a mean of zero and variances to be estimated. We allowed the intercept and static allometric slope to vary among species by adding species random effects, μ_{0j} and μ_{wj}. These random effects are assumed to follow a normal distribution with a mean of zero and variances σ_{0j}^2 and σ_{wj}^2, respectively. These two variances estimate the variability between

species in the two parameters of the static allometric relationship. We also fitted simplified versions of Equation (2) where we removed μ_{wj}, i.e., we constrained the static allometric slopes to be the same for all species and both μ_{0j} and μ_{wj}, that is, constrained slopes and intercepts to be the same for all species.

A slight re-parameterization of the model in Equation (2) allowed us to test whether the evolutionary allometric slope differed from the mean static allometric slope (see van de Pol and Wright 2009):

$$l_{ij} = (\beta_0 + \mu_{0j}) + (\beta_\omega + \mu_{\omega j})b_{ij} + (\beta_b - \beta_\omega)b_{\cdot j} + \epsilon_{ij} \quad (3)$$

In this model, the coefficient for $b_{\cdot j}$ estimates the difference between the mean static allometric slope and the evolutionary allometric slope, that is, $\beta_b - \beta_w$.

We excluded *R. gigas* from the main analysis because it was a clear outlier in the between-species comparison. This aberrant species has the largest body size in the genus, emerges at different times of the year, unlike the other Cape species (Whitehead and Steiner 2001) and is sister to all other species (Kahnt et al. 2017); although for completeness we do provide model results including *R. gigas* in the Supporting Information (Fig. S4). We also checked whether our results were affected by phylogenetic non-independence by fitting models where the residuals ϵ_{ij} were structured assuming either a Brownian motion or Ornstein-Uhlenbeck model for trait evolution (Garland and Janis 1993; Blomberg et al. 2003; Hansen et al. 2008). This analysis was restricted to the 17 species for which we had phylogenetic data (Kahnt et al. 2017) and used functions from the R packages *phylolm* (Ho and Ane 2014) and *geiger* (Harmon et al. 2008).

Allometry and selection imposed by floral oil host plants

We assessed the relationship between the allometric parameters in oil-collecting bees (Tables 1 and 2) and selection imposed by short- to long-spurred, oil-secreting host flowers. We do this across species, and in the case of *R. neliana*, across populations using their population-specific host plants (SI §10).

We estimate the proportion of variance of the allometric intercept that is explained by spur length, after accounting for body size, for all the *Rediviva* species by adding log(spur length) to the model described in Equation 2 and quantifying the change in the variance of the random effects associated with the intercept, σ_{0j}^2.

The analyses described so far used foreleg length and body size on the log scale, which is appropriate for estimating the parameters of the narrow-sense allometric relationship (Pélabon et al. 2014). However, if foreleg length responds to selection imposed by floral spur length, one might expect a linear relationship on the arithmetic scale, that is, a 1 mm change in spur length should lead to a 1 mm change in foreleg length. We therefore also fitted the multi-level models to foreleg length and body size on the arithmetic scale, adding spur length as a covariate.

Results

ESTIMATED PARAMETERS FOR THE STATIC ALLOMETRIC RELATIONSHIPS

There was interspecific variation in both the allometric intercept and slope (F $_{(24, 819)} = 6.08$, $P < 0.001$; Fig. 2, Table 1) and the model explained 96.6% of the variation in foreleg length. However, the variance in slopes among species was 0.076, which was smaller than the mean of the squared standard error of the species-specific slope estimates of 0.108 suggesting that the observed variation in slopes was largely due to sampling variance. The static allometries for all species were negative (slope < 1), except for *R. neliana* that was positive (slope > 1) (Table 1). *Rediviva gigas* was a clear outlier in that it was by far the largest species yet had relatively short legs (see Fig. S1). We excluded this species from the main subsequent analysis.

DIFFERENCE IN STATIC AND EVOLUTIONARY ALLOMETRY

The multi-level model described in Equation 3 also supported the result that there was considerable variation in allometric intercepts among species but no clear evidence for variation in slopes. The residual standard error increased only slightly, from 0.057 to 0.061, when we removed the random slope effect μ_{wj} but increased markedly to 0.238 when we removed the random intercept effect μ_{0j}. The model including all random effects was, however, best supported by AIC (SI §4).

The evolutionary allometric slope estimated by the best-supported model was 2.279 (se $= 0.595$), which is significantly greater than 1 ($t = 2.15$, $df = 22$, $P = 0.04$). Mean body size explained 28% of the variance in the foreleg length among species (see SI §4). This suggests that body size may constrain foreleg length among certain species of *Rediviva*. The evolutionary allometric slope was significantly steeper than the mean static allometric slope (estimated difference 1.74, 95% confidence interval 0.49 to 2.99, Table S2).

Including the outlier species *R. gigas* led to a shallower estimated evolutionary allometric relationship with a slope that was no longer significantly different from 1 and from the mean static allometric slope (SI §6). However, Figure S4 shows that when including *R. gigas* the fitted evolutionary allometric slope is a poor description of the general relationship between body size and foreleg length across species (Table S3). In addition, when accounting for phylogeny the slope estimate of 2.141 (SE $= 0.717$) was similar to the one obtained earlier from the model assuming no phylogenetic dependence structure (2.279, SE $= 0.595$,
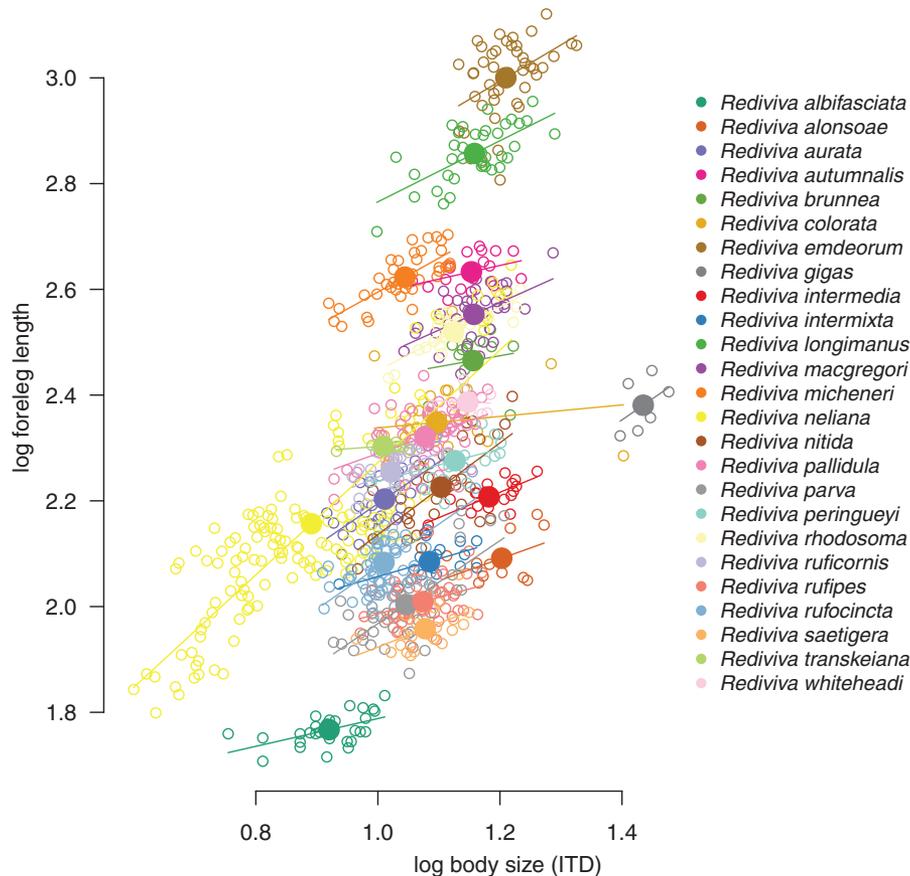
Figure 2. Allometric relationship of leg length against body size of 24 species of female *Rediviva* (excluding *R. gigas*). Each open circle, color coded (using palettes from the *RColorBrewer* (Neuwirth 2014) package), is one observation per species. Each solid circle represents each species mean. The black line shows the fitted evolutionary allometric relationship.

SI §7). We conclude that our earlier results were not sensitive to violation of the assumption that the errors were phylogenetically independent.

EVOLUTIONARY AND STATIC ALLOMETRY OF *R. NELIANA* POPULATIONS

The allometric patterns among *R. neliana* populations were all qualitatively the same as the ones among *Rediviva* species. All effects went in the same direction, even though some were weaker and not statistically supported. There was interpopulation variation in both the allometric intercept and slope ($F_{(9, 127)} = 2.88$, $P < 0.01$; Fig. 3, Table 2) and the model explained 97.6% of the variation in foreleg length. As seen for the species analysis, the observed variation in slopes was largely due to sampling variance; the variance in slopes among populations was 0.048, which was only marginally larger than the mean of the squared standard error of the population-specific slope estimates of 0.023. Despite finding that overall *R. neliana* has a steep evolutionary allometric slope (Fig. 2), we found that the static allometric slope for

almost all populations was negative, with three populations close to isometry (Table 2, Fig. 3).

From the variable slopes model, the estimated evolutionary allometric slope across *R. neliana* populations was 1.259 (SE = 0.282), which was not significantly different from 1 ($t = 0.91$, $P = 0.4$). Mean body size explained 67.8% of the variance in the foreleg length among populations (SI §8). This suggests that there is only a small amount of independent variation in the trait relative to body size across populations of *R. neliana* (Fig. 3). The evolutionary allometric slope was steeper than the mean static allometric slope, but the confidence interval of the model estimating the differences overlapped zero (estimated difference 0.49, 95% confidence interval −0.17 to 1.15, Table S5) indicating less statistical support for differences between the static and evolutionary allometry for *R. neliana* populations.

RELATIONSHIP BETWEEN ALLOMETRY AND SELECTIVE ENVIRONMENT

Spur length on the log scale explained 36.4% of the remaining variance, after accounting for body size, in the intercept among
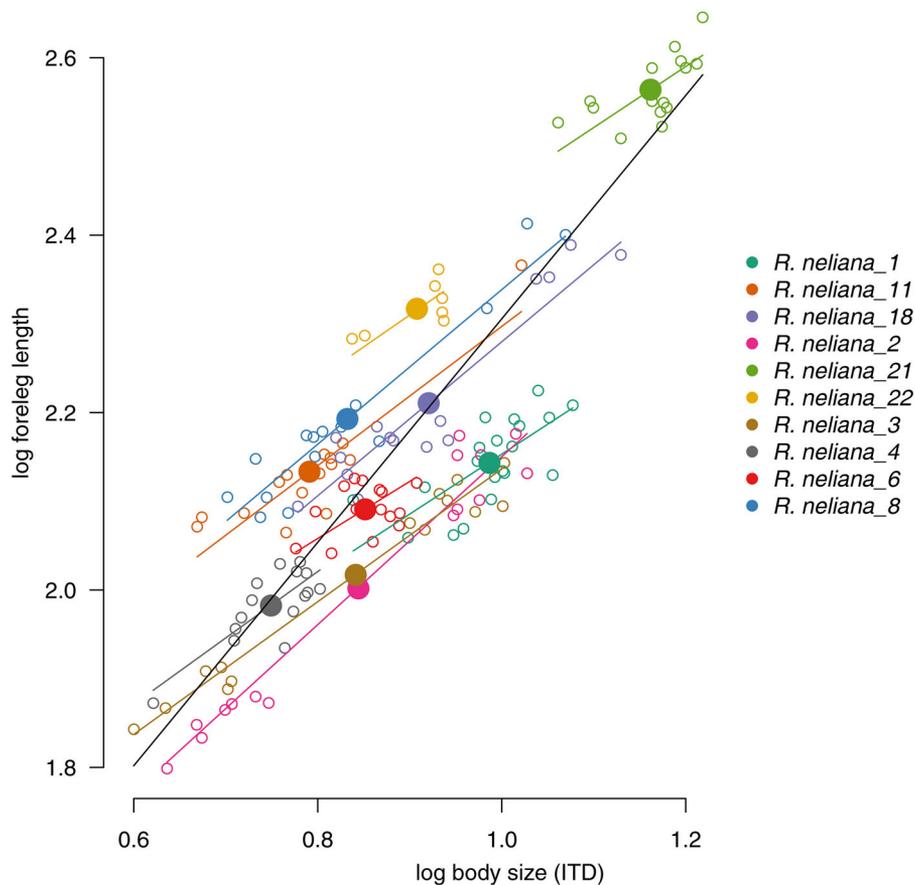
Figure 3. Allometric relationship of foreleg length against body size in *R. neliana* populations. The black line shows the fitted evolutionary allometric relationship. Populations correspond with site number in Table 1 in Steiner and Whitehead (1990).

species when added as an explanatory variable to the model in Equation 3 (Fig. 4A; SI §9). When analyzing allometry on the arithmetic scale, spur length explained 49.4% of the variance in the intercept among species after accounting for body size. The estimated effect of the coefficient for spur length was 0.51, which is considerably less than 1 ($t = -4.58$, $P < 0.001$; Fig. 4B).

For *R. neliana*, when assessing the relationship between populations and spur length on the log scale using multilevel models, we found that spur length explained almost all (94.3%) of the remaining variance, after accounting for body size, in the intercept among populations (Fig. 4C; SI §10). When analyzing allometry on the arithmetic scale, spur length explained 94.9% of the variance in the intercept among species after accounting for body size. The estimated effect of the coefficient for spur length was 0.31, which is considerably less than 1 ($t = -25.59$, $P = 0$, Fig. 4D).

Discussion

We quantified both static and evolutionary allometry to explore adaptation or constraint in a pollinator displaying exaggerated

traits. By estimating narrow-sense allometric parameters we were able to test these opposing hypotheses and the role of allometry in shaping foreleg variation in oil-collecting bees. Our results suggest that static slopes are less evolvable and are under stabilizing selection, whereas the static intercept can evolve more freely, and its changes are driven by variation in body size and spur length of host plants.

We found a lack of evidence of substantial variation in static slopes among species and populations of *Rediviva*. This result fits well with the constraint hypothesis because the static slopes seem to have a rather low rate of evolution (Voje and Hansen 2013; Firmat et al. 2014; Pélabon et al. 2014; Voje et al. 2014; Bolstad et al. 2015; Voje 2016; Houle et al. 2019). However, our static slopes across species and populations did not align particularly well with evolutionary allometry, which can be argued as evidence against the allometric-constraint hypothesis (Firmat et al. 2014; Pélabon et al. 2014; Voje et al. 2014; Voje 2016). Further evidence against the constraint hypothesis was seen in the substantial variation recorded in the static intercept across species and populations. Changes in the intercept are an important source of morphological evolution (Gould 1966; Cheverud 1982; Emlen
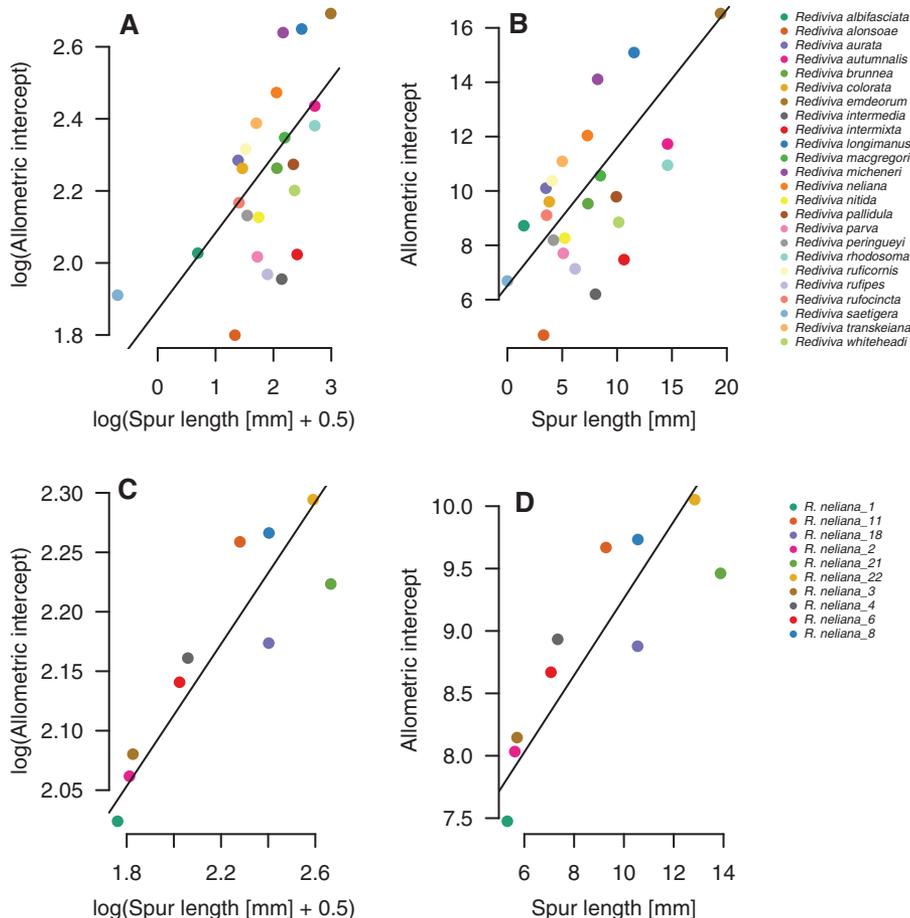
Figure 4. Relationship of allometric intercept to spur length of the oil host plants for *Rediviva*. Plots (A) log-log scale and (B) arithmetic scale of *Rediviva* species. For *R. neliana* populations, plots (C) log-log scale and (D) arithmetic scale.

and Nijhout 2000; Frankino et al. 2005; Toju and Sota 2006; Egset et al. 2012; Pélabon et al. 2014); as we see here for our study system. However, at the population level, variation in static intercept was linked to body size, with body size explaining a high percentage (∼68%) of foreleg variation, that is, evidence of a developmental constraint (Lande and Arnold 1983; Shingleton et al. 2007; Voje and Hansen 2013; Pélabon et al. 2014). The variation in the static intercept was also related to some degree to the variation in spur length of the host plants, that is, providing support for the adaptive hypothesis. At the population-level spur length explained almost all of the remaining variance seen in static intercepts on both the log and arithmetic scales (see also Steiner and Whitehead 1990, 1991). Across species, we found body size explained a small percentage (28%) of foreleg variation, indicating that foreleg length is able to change relatively free of body size in some species (Cheverud 1982; Zeng 1988; Higginson et al. 2015; Tidière et al. 2017). This pattern may be attributed to the different oil-collecting behaviors of species. For example, *R. pallidula* foreleg length varies independently of body size, whereas, in *R. rufocincta*, foreleg variation appears con-

strained by body size (Steiner and Whitehead 1991). Spur length explained ∼36% on the log scale and ∼49% on the arithmetic scale of the remaining variance in the intercept among species (but see Pauw et al. 2017). Although these results indicate a positive relationship between foreleg and spur length, we found little support where foreleg length is tracking mean spur length in a one-to-one relationship. Assessing the relationship between static intercept and spur length on the arithmetic scale allowed us to test for such an adaptive hypothesis; on the log-log scale one cannot interpret the slope of such a relationship with respect to an adaptive hypothesis. That we found little support, could be attributed to our measure of foreleg length that may not necessarily capture a one-to-one type relationship. Placement of oil-absorbing hairs on the foreleg varies across species (Steiner and Whitehead 1991; Whitehead and Steiner 2001; Kuhlmann and Hollens 2015) and a better assessment of how foreleg length is shaped by host plant selection may be to relate the position of the oil absorptive hairs on the foreleg with the position of oil glands inside the spur. We also note that our analysis could not account for sampling variation in spur length and the estimated slope is most likely

shallower than the true slope (Hansen and Bartoszek 2012). We do not have the necessary data to correct our estimate for sampling variance in spur length and generic statistical methods that are often used when there is sampling variance in the explanatory variable should not be used for evolutionary regressions (Hansen and Bartoszek 2012). Our data therefore indicate a slope that is possibly closer to 1 than what our estimate suggests.

The high variability/evolvability of the intercept appears to be influencing the evolution of static allometry in *Rediviva* likely owing to the selection generated by the spur length of the host plants. This adaptive variation in static allometric intercept has generated an evolutionary allometry steeper than the static allometry among species and populations (Klingenberg and Zimmermann 1992; Voje and Hansen 2013; Pélabon et al. 2014; Tidière et al. 2017). The finding of steeper evolutionary allometry among species is not surprising (Pélabon et al. 2014; Voje et al. 2014). However, the steeper evolutionary allometry among populations is somewhat surprising considering that at the microevolutionary scale static allometries usually predict evolutionary allometries (Firmat et al. 2014; Voje et al. 2014). One possible explanation relates to sampling error of the static slopes; we found sampling variance explained quite a bit of the variation in static slopes (SI §8). An alternative possibility is that the populations may be evolving as separate lineages ("incipient species") (Whitehead and Steiner 1992; Kahnt et al. 2018) and therefore a difference between static and evolutionary allometry could be expected. Strong ecological and morphological differences seen across *R. neliana* populations provide support for this argument. *Rediviva neliana* shows a wide distribution across different and discontiguous habitats, where populations visit different host plant species (Steiner and Whitehead 1990; Whitehead et al. 2008). The large variation in foreleg length of *R. neliana* populations, up to almost double, is also unique amongst *Rediviva* species.

At first sight, our results of low variation in static slopes among species and populations suggest that the evolution of the allometric slope appears constrained; however, nearly all static slopes were <1 (Tables 1 and 2). One possible explanation is that foreleg length may be under stabilizing selection favoring shallower slopes (Eberhard 2009; Pélabon et al. 2011). Interacting traits may either require a precise fit, or it may be beneficial to have an optimal foreleg length that fits the pattern of distribution of spur lengths of host plants (Berg 1959, 1960; Eberhard et al. 1998; Armbruster et al. 2009; Eberhard 2009; Pélabon et al. 2011). Under these scenarios, this would likely generate a decoupling of the variation in foreleg length and body size. As discussed above, we see that adaptation to host plants with different spur length can be realized among species by a change in foreleg length independent of body size (i.e., change in static intercept). Whereas, at the population level, we note that adaptation to host plants requires a correlated change in body size with a change in

static intercept. This may be more pronounced at the population level due to the short time scales over which intercepts have been able to diverge (Pélabon et al. 2014; Voje et al. 2014).

Another type of stabilizing selection may relate to securing functional size relationships (Frankino et al. 2005; Pélabon et al. 2014; Houle et al. 2019). There may be size trade-offs in *Rediviva* with other functional traits involved in foraging (Melin et al. 2019), nesting behavior (Melin and Colville 2019), or the size of pollen-carrying hind legs (Martins et al. 2014; Helm et al. 2017). Pollinators displaying exaggerated traits exhibit functional costs in terms of increased handling time of host flowers (e.g., Karolyi et al. 2013; Bauder et al. 2015). *Rediviva* are ground-nesting bees, and although direct observation is missing, the female bees, like most ground-nesting solitary bees, use their forelegs to excavate nests (Danforth et al. 2019). This nesting behavior may place other selection pressures on foreleg morphology. The unexplained variance seen for foreleg lengths after accounting for body size and spur length across species may relate to such pressures and highlights the need to obtain additional data on oil-bee biology to more fully understand the drivers associated with the evolution of their forelegs.

In summary, static slopes across species and populations of *Rediviva* bees seem to have low evolvability, and for bees to adapt their forelegs to the spur length of their host plants, they either change their static intercept or their body size, or a combination of the two. The shallow slopes found among species and populations suggest a third possibility of stabilizing selection generating foreleg length independent of body size. These findings suggest that across *Rediviva* species the developmental process is not conserved (e.g., Higginson et al. 2015; see also SI §7) and that the scaling relationships themselves are undergoing some form of adaptive evolution, i.e., are potential targets of selection (Emlen and Nijhout 2000).

AUTHOR CONTRIBUTIONS

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DATA ARCHIVING

The data are available from the University of Cape Town's data portal (DOI: 10.25375/uct.13297640). The code needed to reproduce all analyses in this manuscript will be available at: https://github.com/resaltwegg/Rediviva-allometry

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Summary of morphological data and allometric parameters for 25 species of *Rediviva* bees.

Table S2: Difference (delta) between the evolutionary and static allometric slopes estimated by three different models across *Rediviva* species.

Table S3: Difference (delta) between the evolutionary and static allometric slopes estimated by three different models across *Rediviva* species including *R. gigas*.

Table S4: Summary of morphological data and allometric parameters for 10 populations of *R. neliana* bees.

Table S5: Difference (delta) between the evolutionary and static allometric slopes estimated by three different models across *R. neliana* populations.

Figure S1: Allometric relationship of leg length against body size in *Rediviva* species.

Figure S2: Allometric relationship of leg length against body size in *Rediviva* species.

Figure S3: Allometric relationship of leg length against body size in *Rediviva* species, excluding *R. neliana*.

Figure S4: Allometric relationship of leg length against body size in *Rediviva* species, including *R. gigas*.

Figure S5: Allometric relationship of leg length against body size in *R. neliana* populations.

Figure S6: Leg length of *Rediviva* individuals of different species against mean spur length of their host plant flowers.

Figure S7: Relationship of allometric intercept to spur length of the host plants for *Rediviva* bee species.

Figure S8: Relationship of allometric intercept (i.e. estimated species-specific leg length from Model m11) to spur length of the host plants for *Rediviva* bee species, fitted on the aritmetic scale.

Figure S9: Leg length of *R. neliana* individuals from different populations against spur length of their host plant flowers.

Figure S10: Relationship of allometric intercept to spur length of the host plants for *Rediviva neliana* populations.

Figure S11: Relationship of allometric intercept (i.e. estimated population-specific leg length from Model m11) to spur length of the host plants for *Rediviva neliana* populations, fitted on the aritmetic scale.

Supporting Information