Evolutionary genetics of a bizarre adaptation: elongated forelegs of South African *Rediviva* bees as an adaptation to their *Diascia* flower hosts

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To: Everyone, who inspired me:

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General Introduction

Coevolution

The evolution of ecological interactions has fascinated scientists for generations. The idea that ecologically closely interacting taxa might reciprocally influence each other's evolution is as old as Darwin's ground-breaking 'On the origin of species', where he acknowledged species interactions as major driver of adaptive evolution and diversification (Darwin, 1859): ' I can understand how a flower and a bee might slowly become, either simultaneously or one after the other, modified and adapted in the most perfect manner to each other, by continued preservation of individuals presenting mutual and slightly favourable deviations of structure.' Later on, Darwin pointed out the first potential example for a coevolutionary interaction, when he hypothesised that the long-spurred Malagasy star orchid *Angraecum sesquipedale* must be pollinated by a hawk-moth with an exceptionally long tongue (Darwin, 1862; Fig. 1). This pollinator species (*Xanthopan morganii praedicta*) was unknown to Darwin and only first described in 1903 (Rothschild & Jordan, 1903). Nevertheless, subsequent studies proposed that a pollinator shift model (Grant & Grant, 1965; Stebbins, 1970), where the plant has 'shifted' to the moth as new pollinator and adapted to its pre-existing long tongue, rather than reciprocal coevolution might be more likely to drive long spurs in *A. sesquipedale* (Wasserthal, 1997; Whittall & Hodges, 2007). The first ecological study to explicitly study coevolution was carried out by Ehrlich & Raven (1964), who investigated the interactions between butterfly herbivores and their host plants and hypothesised that the evolution of plant defences followed by counter-adaptations in its herbivores (i.e. coevolution) might result in adaptive radiation (escape-and-radiate-hypothesis). An increasing interest in the topic and increasing misuse of the term 'coevolution' necessitated a formal definition; Janzen (1980) defined 'coevolution' as an evolutionary change in a trait of the individuals in one population in response to a trait of the individuals of a second population, followed by an evolutionary response by the second population to the change(s) in the first. One of the first and most frequently cited books on coevolution by Futuyma & Slatkin (1983) appeared only a few years later and laid out the fundaments for the concept of 'coevolution' by, firstly, providing a theoretical framework and, secondly, analysing coevolution 'in action' in various systems.

Thompson's hypothesis of a geographic mosaic of coevolution (Thompson, 1994, 2005) further revolutionized the field of coevolution research. According to Thompson (1994, 2005), interacting populations are connected to each other in a network called the geographic mosaic, which varies in space and time. At a specific point in time, some populations within the mosaic may be involved in a close interaction with reciprocal adaptations, so-called coevolutionary 'hot spots', while others may not—coevolutionary 'cold spots' (Thompson, 1994, 2005). Interaction outcomes are thus not only

influenced by processes that act within populations (selection, drift, local extinction etc.), but also by processes occurring between populations (dispersal, migration), whereby traits in a hot spot may spread to populations with different selection regimes through trait remixing (Thompson 2005). Following Thomson (2005), there is no reason to expect that all populations of the interacting species must obey strict coevolution as defined by Janzen (1980), even though substantial reciprocal evolution might happen across the entire geographic range of interconnected metapopulations. Furthermore, more recent studies highlighted the importance of investigating coevolution in a community network approach as species might even coevolve via indirect interaction effects (Guimarães, Pires, Jordano, Bascompte, & Thompson, 2017).

Systems in which species reciprocally influence each other's evolution, i.e., coevolve, might encompass interactions between hosts and their associated parasites (Hughes et al., 2007), predators and their prey (Brodie et al., 2005), and also mutualistic relationships between hosts and their endosymbionts (McFrederick & Taylor, 2013) or plants and their pollinators (Cruaud et al., 2012). While coevolution between hosts and their associated parasites (e.g. Hamerlinck, Hulbert, Hood, Smith, & Forbes, 2016; Lauron et al., 2015) or symbionts (e.g. Kaltenpoth et al., 2014; Urban & Cryan, 2012) has been explored in much detail, less attention has been paid to plant-pollinator coevolution.

Fig. 1 The first photographical documentation (Wasserthal, 1997) of the pollination of the Malagasy star orchid *Angraecum sesquipedale* by the hawk moth *Xanthopan morganii praedicta*, which was considered as classic example for coevolution between a pollinator (proboscis) and its host plant (spurs).

Ecological adaptation in pollinators and potential coevolution with their host plants

Ecological interactions between different species may lead to specific adaptations within the interaction partners, which are termed co-adaptations when they are due to coevolution (Wade, 2007). Since interactions between plants and their pollinators might have considerable fitness consequences for each partner, adaptations to increase pollination efficiency (plant perspective) or exploitation of floral reward (pollinator perspective) are frequently found (Amrad et al., 2016; Boberg et al., 2014; Cosacov, Cocucci, & Sérsic, 2014; Newman, Manning, & Anderson, 2015; Pauw et al., 2017; Stankowski, Sobel, & Streisfeld, 2017). Pollinator-driven adaptation might even result in bizarre plant morphologies. Elongated spurs, such as in *Platanthera* orchids or in *Calceolaria polyrhiza* herbs, may be due to adaptation to the pre-existing proboscis length of the local pollinator fauna, i.e. pollinator shifst (Boberg et al., 2014; Cosacov et al., 2014), similar to that which has been suggested for the Malagasy star orchid *Angraecum sesquipedale* (Wasserthal, 1997; Whittall & Hodges, 2007). Alternatively, coevolutionary interactions may lead to exaggerated traits such as long spurs in flowers and long tongues in pollinators, described as 'Darwin's race' (Johnson, 2009), where in each generation plants with longer spurs are favoured, which in turn favours insects with even longer tongues. Theoretically, this could lead to infinite trait exaggeration but lengthening is halted due to selective constraints associated with extreme morphologies, e.g. increased energetic costs or flight constraints in the insect (Johnson & Anderson, 2010).

Some of the best available evidence for the occurrence of 'Darwin's race' stems from systems in South Africa (Anderson & Johnson, 2007; Johnson & Anderson, 2010; Pauw, Stofberg, & Waterman, 2009; Steiner & Whitehead, 1990). For example, some nemestrid and tabanid flies adapt to the nectar-secreting long tubes of their host plants by developing an extended proboscis in response to an extended corolla, which in turn evolved in response to the fly's proboscis, e.g. *Moegistorhynchus longirostris* flies & *Lapeirousia anceps* irises (Pauw, Stofberg, & Waterman, 2009) or *Prosoeca ganglbaueri* flies & *Zaluzianskya microsiphon* host plants (Anderson & Johnson, 2007) were suggested to coevolve. However, for other South African pollination systems either it is not clear whether coevolution or pollinator shifts account for long plant spurs (*Prosoeca longipennis* flies-pollination guilt, Newman, Manning, & Anderson, 2014; *Gladiolus longicollis* irises-*Agrius convolvuli* hawkmoths, Alexandersson & Johnson, 2002) or the pollinator shift model is favoured (*Disa draconis*-tabanid and nemestrid flies, Johnson & Steiner, 1997; *Tritoniopsis revoluta*-nemestrid flies, Anderson, Ros, Wiese, & Ellis, 2014). Another peculiar system for which the relative importance of coevolution and pollinator shifts have not been studied yet are long-legged *Rediviva* bees and their long-spurred *Diascia* hosts (Steiner and Whitehead 1990, 1991). In general, it seems that pollinator shifts have occurred frequently and probably more often than coevolution during the evolutionary

history of plant-pollinator interactions (e.g. Boberg et al., 2014; Cosacov, Cocucci, & Sérsic, 2014; Machado, Robbins, Gilbert, & Herre, 2005; Michez, Patiny, Rasmont, Timmermann, & Vereecken, 2008; van der Niet & Johnson, 2012; van der Niet et al., 2014; Whittall & Hodges, 2007), probably because most plant-pollinator interactions are rather asymmetrical with specialists interacting with generalist species, rather than with other specialists, (Bascompte, Jordano, & Olesen, 2006; Vázquez & Aizen, 2004), which reduced the selection pressure to reciprocally adapt and coevolve (Kiester, Lande, & Schemske, 1984).

(Co-) adaptation of the pollinator to its host plant(s) and *vice versa* may also cause ecological speciation. For example, adapations to the host plant may result in strongly divergent selection pressures and impede gene flow between populations using different host plants (Nosil, 2012; Schluter, 2009). When gene flow among locally adapted populations of a pollinator species is reduced, initially a heterogeneous pattern of genomic divergence among neighbouring populations will results, with increased genetic differentiation at loci under divergent selection (those underpinning the traits related to differences in, say, pollinator morphology), i.e. 'islands of genomic divergence', while the remainder of the genome will not exhibit strong differentiation (e.g. Nosil, Funk, & Oritz-Barrientos, 2009; Wu, 2001). Increased genetic differentiation at selected loci might also promote reproductive isolation via pleiotropic gene effects or when the selected loci are closely linked to loci affecting reproductive isolation, i.e. linkage disequilibrium, (Nosil, Funk, & Oritz-Barrientos, 2009; Rundle & Nosil, 2005). Once barriers to gene flow exist between locally adapted populations of a pollinator, genome-wide neutral differentiation may increase over time such that genomic islands of divergence will increase, creating genomic continents of divergence (Feder, Egan, & Nosil, 2012). Therefore, the more adaptively divergent populations become, the less gene flow and thus the more genome-wide differentiation will build up, yielding a pattern of isolation by adaptation (IBA, Feder, Egan, & Nosil, 2012). If finally populations are sufficiently genetically differentiated and are no longer able to reproduce with each other, the speciation processes is completed. Hence, host plant adaptation might result in ecological speciation of the pollinator and even cause the plant to simultaneous speciate if reduced gene flow between pollinator populations also reduces gene flow between their host plants.

Coevolution and cospeciation

Providing convincing support for coevolution between interacting species is challenging since several lines of evidence are required (Anderson, 2015). It is not sufficient to show correlated trait change in the interacting partners but also that each taxon is exerting selection on the trait of its partner, resulting in a fitness benefit and trait change due to the mutual interaction (Anderson, 2015). Testing for coevolution becomes even more complicated when there is no strict one-to-one but rather diffuse

coevolution, which is usually the case for most pollinator- plant- interactions as they comprise many interacting partners and asymmetric specialisations (Bascompte & Jordano, 2007; Vázquez & Aizen, 2004). Given these difficulties, cophylogenetic studies comparing the phylogenies of interaction partners, became a promising approach to study coevolution since cospeciation, i.e. congruent speciation events, was hypothesised to indicate coevolution (Page, 2003). However, cospeciation does not provide unequivocal support for coevolution since a one-to-one match of speciation events (cospeciation) might be also due to simultaneous speciation in response to the same biogeographic events (vicariance) experienced by the interaction partners (Janz, 2011; Poisot, 2015; Segraves, 2010) or due to phylogenetic tracking, in which one species tracks the speciation events of its interaction partner (Althoff, Segraves, & Johnson, 2014; Suchan & Alvarez, 2015; Fig. 2). Furthermore, coevolution will only lead to cospeciation when the coevolutionary interaction occurs over a sufficient time period and when the coevolutionary interaction also triggers reproductive isolation between populations and thus decreases gene flow and finally causes incipient speciation. Hence, coevolution might well occur without cospeciation, e.g. when coevolutionary interactions are restricted to short evolutionary time periods (Janz, 2011). In addition, coevolution in 'escape-and-radiate' interactions, such as in the plant-herbivore-system analysed by Ehrlich & Raven (1964), are not expected to lead to parallel speciation but rather bursts of speciation events followed by lag phases that alternate in their timing between the coevolving lineages (Thompson, Segraves, & Althoff, 2017). Thus, coevolution might only result in cospeciation in rare cases and the terms 'cospeciation' and 'coevolution' should therefore not be used synonymously.

These rare instances when coevolution might result in cospeciation include interactions between hosts and their vertically transmitted parasites or interactions between hosts plants and their pollinating floral parasites (brood pollination mutualisms) that control the movement of host gametes (Thompson et al., 2017). Prominent examples for the latter are yucca (*Yucca*) & yucca moths (*Tegeticula*, *Parategeticula*), figs (*Ficus*) & fig wasps (Agaonidae, Chalcidoidae), leafflower trees (*Glochidion*) & leafflower moths (*Epicephala)* and woodland stars (*Lithophragma*) & *Greya* moths (Hembry et al., 2014; Segraves, 2010). In these obligate pollinating seed-predation mutualisms the plants have no alternative pollinator as do the pollinators have no alternative host for reproduction, suggesting a tight evolutionary coupling is highly likely to evolve (Hembry et al., 2014). Although former studies (Godsoe, Yoder, Smith, Pellmyr, & Wilbur, 2008; Weiblen & Bush, 2002) suggested a high frequency of cospeciation in this putatively coevolving systems, more recent studies support a prevalence of host shifts (Kawakita, Takimura, Terachi, Sota, & Kato, 2004; Machado, Robbins, Gilbert, & Herre, 2005) or cospeciation due to vicariance (Althoff, Segraves, Smith, Leebens-Mack, & Pellmyr, 2012) for these mutualisms. Hence, when even these highly specialised coevolved interactions show little evidence for cospeciation driven by coevolution, speciation due to coevolution might indeed be rare in

nature and may occur only in concert with other processes (Hembry et al., 2013). Nevertheless, limited studies have investigated the relationship between coevolution and diversification (Hembry et al., 2013) and thus additional studies are required to support the conclusion that cospeciation is infrequently the result of coevolution. Hence, cophylogenetic analyses in combination with ecological studies showing reciprocal trait change between interacting populations (see above) and reproductive isolation due to the coevolutionary interaction are necessary to test if coevolution drives cospeciation. Such studies are particularly interesting in biodiversity hotspots that due to there high diversifity and specialisation of biotic interactions posses a great potential for coevolution to occur.
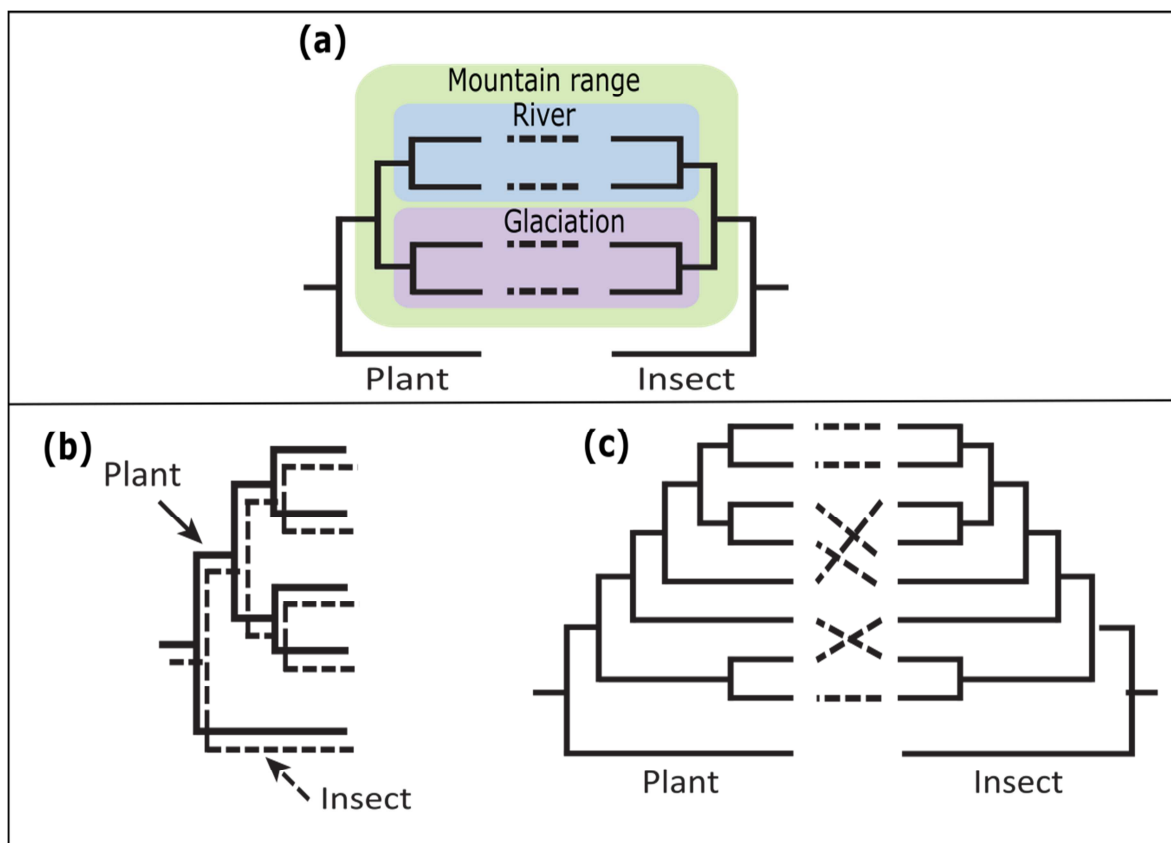

Fig. 2 Several processes might result in phylogenetic congruence: (a) vicariance events, (b) phylogenetic tracking or (c) cospeciation (modified from Althoff, Segraves, & Johnson, 2014).

Pollinator and plant biodiversity in the Cape biodiversity hotspot

The Greater Cape Floristic Region (GCFR) of southern Africa exhibits a plethora of plant and pollinator species and a high degree of ecological specialization, leading to peculiar pollination syndromes such as long-proboscid flies or oil-collecting bees (Goldblatt & Manning, 2002; Steven Johnson, 2010). The GCFR is considered a global biodiversity hotspot (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000) and its richness and endemism of plant species is ranked

second-highest in the world (Kier et al., 2009). Worldwide the GCFR and SW Australia are the only regions where centres of plant and bee diversity overlap (Kuhlmann 2005; Linder *et al.* 2010). Within the Cape, the winter rainfall area is the region with the highest number of bee species, with nearly half of the species being endemic to this area (Kuhlmann, 2009). The characteristic vegetation types of the GCFR are Fynbos and Succulent Karoo (Born *et al.* 2007), the latter being largely congruent with the winter rainfall area and comprising the world's richest succulent flora (Lombard *et al.* 1999).

Historically, stable climatic conditions during the Miocene might have resulted in low extinction rates and thus contributed to radiation of the GCFR flora (Cowling et al., 2009; Neumann & Bamford, 2015; Schnitzler et al., 2011). In general, Mediterranean-like climates such as those experienced by the GCFR (Goldblatt & Manning, 2000) might have been buffered against climate extremes for various reasons and thus they exhibit comparably low extinction rates (Cowling et al., 1996; Goldblatt & Manning, 2002). Moreover, the GCFR is characterised by steep climatic, topographic and soil gradients (Ellis, Anthony Verboom, van der Niet, Johnson, & Peter Linder, 2014; Goldblatt & Manning, 2002), generating environmental heterogeneity and potentially driving ecological speciation. Pollinator-mediated selection might also be a key component of Cape plant diversity (Johnson & Steiner, 2003; Johnson, 2010; Pauw et al., 2009; Valente, Manning, Goldblatt, & Vargas, 2012; van der Niet & Johnson, 2009; van der Niet et al., 2014). One mechanism for how pollinators drive plant divergence might be via spatial gradients in pollinator availability that select for traits enabling exploitation of alternative pollinators, i.e. pollinator shifts (Ellis et al., 2014). Several studies have suggested the existence of spatial gradients of pollinator availability and associated divergence in floral traits and pollinator use (Johnson, 1997; Johnson & Steiner, 1997; Pauw et al., 2009; Waterman et al., 2011). However, the exact drivers of phytodiversity in the GCFR are still controversially discussed (Ellis et al., 2014; Schnitzler et al., 2011; Verboom et al., 2009), and pollinator-driven diversification of the local flora seem to matter as well as climatic and edaphic factors.

Considering pollinators, their diversification may also be driven by reduced gene flow due to the region's geology and climate (Kahnt, Soro, Kuhlmann, Gerth, & Paxton, 2014; Linder et al., 2010). Unfavorable climate (cold, rain, wind) during the peak flowering season strongly reduces the daily activity of foraging bees (Kuhlmann, unpublished) and might select for increased foraging and nesting efficiency, resulting for example in small body sizes (smaller bees are able to carry more pollen relative to their body mass; Linder et al., 2010). A smaller body size in winter-active bees also reduces flight distance, which in turn may reduce gene flow between the bees but also between the plants whose pollen the bees carry, thus facilitating speciation in both (Linder et al., 2010). Host plant adaptation might be another driver of pollinator diversification (Pauw et al., 2009), where plant and pollinator evolution in the GCFR might be linked unidirectional, i.e. the pollinator or host plant

'shifts' and adapts to pre-existing traits of its interaction partner, or bidirectional, i.e. coevolution (Johnson & Anderson, 2010). A recent global review suggested that host plant shifts play a major role in insect speciation (Forbes et al., 2017). Still, while there is accumulating evidence for pollinator-driven speciation in Cape plants (van der Niet & Johnson, 2009; van der Niet et al., 2014), there is only limited evidence for the reverse, i.e. host plant-driven speciation of Cape pollinators (Ellis et al., 2014). Thus, further studies are required to address the role of plant-mediated selection for pollinator adaptation and speciation and how pollinators might in turn affect the diversification of their plants.

South African *Rediviva* bees and elongated forelegs

Bees of the South African genus *Rediviva* (family: Melittidae) are fascinating examples of species possessing a bizarre morphology that might result from pollinator-plant coevolution. *Rediviva* bees are solitary, ground nesting (Kuhlmann, 2014) and strictly endemic to South Africa and Lesotho, with the majority of species (15 out of 26 described species) occurring in the winter-rainfall area of South Africa (Kuhlmann, 2012; Whitehead & Steiner, 2001; Whitehead, Steiner, & Eardley, 2008). *Rediviva* belongs to a small group of oil-collecting bee taxa (Apidae: Centridini, Tetrapedini, Ctenoplectrini, Tapinotaspini; Buchmann, 1987; Cocucci, Sersic, & Roig-Alsina, 2000; Houston, Lamont, Radford, & Errington, 1993; Steiner & Whitehead, 2002 and Melittidae: *Macropis*, *Rediviva*; Michez et al. 2008), in which female *Rediviva* exhibit remarkably long forelegs for accessing oils within the elongate floral spurs of their principal host plants (Vogel 1974, 1984; Steiner and Whitehead 1990, 1991). Apart from *Rediviva*, elongated legs for oil-collecting purposes are only known in *Centris hyptidis* (Apidae, Machado, Vogel, & Lopes, 2002) and *Tapinotaspis* species (Apidae, e.g. Cosacov, Nattero, & Cocucci, 2008). The main oil hosts for *Rediviva* belong to the genus *Diascia* (family: Scrophulariaceae), which comprises around 73 species (Steiner, 2011) and is characterised by oil-secreting twin spurred flowers (Steiner, 2011). Annual *Diascia* species occur exclusively in the winter rainfall area while perennial *Diascia* species are restricted to the summer rainfall area of South Africa (Hilliard & Burtt, 1984). Female *Rediviva* bees collect floral oil by inserting the forelegs into the flower spurs, rubbing against the spur walls and absorbing floral oil with specialised tarsal pubescence (Vogel 1984; Steiner & Whitehead 1988, Fig. 2). The collected oil is used for larvae provision and probably to make the underground nest (brood cells) waterproof (Kuhlmann, 2014; Pauw, 2006). During oil-collection, pollen attaches to specific parts of the bee's body (Steiner & Whitehead, 1988) and is transported to the next flower. *Rediviva* thus significantly contributes to *Diascia* pollination since most *Diascia* are self-incompatible and mainly pollinated by *Rediviva* (Steiner & Whitehead, 1988). Consequently, *Rediviva* and *Diascia* might exert strong reciprocal selection on each other as their interaction has major fitness consequences for both.
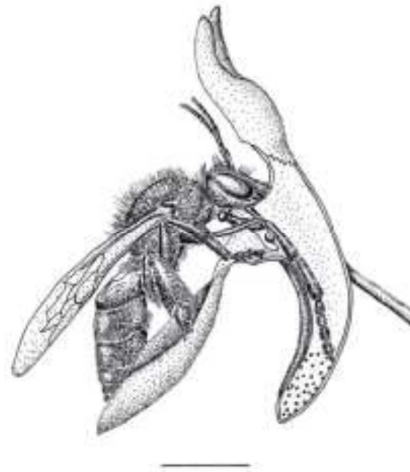
Fig. 3 *Rediviva* species collecting oil on *Diascia*. Photo (left): with kind permission of W. Hattingh, Drawing (right): Steiner & Whitehead (1990).

Since the oil-secreting hairs (trichome elaiophores) of *Diascia* spp. are usually concentrated in the bottom of the spurs (but see: Hollens, van der Niet, Cozien, & Kuhlmann, 2016), the bees might experience strong selection pressure on fitting, i.e. long enough, legs to be able to successfully collect oil. For plants, on the other hand, individuals with spurs slightly longer than the forelegs of the pollinators might have a fitness advantage compared to congeners with shorter spurs due to increased handling times and thus higher likelihood of being successfully pollinated (Steiner & Whitehead, 1990). Pollinator-mediated selection might drive the evolution of long spurs, which in turn might select for even longer legs in the bee (Steiner & Whitehead, 1990). Such reciprocal morphological adaptations as well as synchronized phenologies might reinforce plant-pollinator specialisation and thus generate the potential for coevolution (Kawakita & Kato, 2017). Indeed, *Rediviva* shows not only interspecific variation in female foreleg length, FLL, (*R. albifasciata*: 6.5mm to *R. emdeorum:* 26 mm; Whitehead and Steiner 2001) but also intraspecific FLL variation that seems to be significantly correlated with the spur length of the *Diascia* host plants (Hollens et al., 2016; Steiner & Whitehead, 1990, 1991), indicative of selection for trait matching. Yet demonstrating a correlation between FLL and spur length is not sufficient to conclusively show coevolution in *Rediviva-Diascia*, as such a correlation might equally result from pollinator shifting.

Moreover, as most *Rediviva* are not specialised on a single host but rather use a range of *Diascia* species (Whitehead & Steiner, 2001; Whitehead et al., 2008) or other plant species from the Scrophulariaceae, Orchidaceae, Stilbaceae and Iridaceae as sources of oil (Kuhlmann & Hollens, 2015; Pauw, 2006; Steiner & Whitehead, 1988, 2002; Waterman et al., 2011), FLL evolution might be rather driven by the spur length of the local community than by a single host plant (Pauw et al., 2017, Hollens-Kuhr et al., unpublished). Consequently, if *Rediviva* and *Diascia* are coadapting then

coevolution is rather diffuse and likely represents a geographic mosaic, with only some population pairs coevolving but not all (Thompson, 2005). This geographic mosaic hypothesis is supported by studies on long-tubed flowers and their long-proboscid flies in the GCFR (Anderson & Johnson, 2007, 2009; Pauw et al., 2009) and by analyses of *Rediviva -Diascia* communities in the winter rainfall area which revealed a trait match, i.e. FLL and spur length, at only some sites (Hollens-Kuhr et al., unpublished).

Aims

So far, nothing is known about the evolutionary genetics of the *Rediviva- Diascia* relationship. In order to obtain a better understanding of the evolution of this plant-pollinator interaction it would be important to address the evolution and genetic underpinning of one of the key traits involved in this relationship: the elongated forelegs in *Rediviva*. Furthermore, another interesting question in this regard is whether and how FLL variation impacts the genetic structure of *Rediviva* populations and diversification processes of the whole genus. If FLL is indeed under substantial selection imposed by the host plant, then *Rediviva* populations might become locally adapted and strongly differ in their FLL and show increased genetic differentiation at the loci underlying FLL ('islands of divergence'). Moreover, if the loci underlying FLL have pleiotropic effects or are in linkage disequilibrium with loci regulating reproductive behavior, gene flow might not only be locally reduced at the genes controlling FLL but genome-wide between populations that vary in FLL. Significant genetic differentiation might build up over time, eventually leading to completely reproductively isolated species once populations are no longer able to interbreed. Thus, adaptation to different host spur lengths might lead to ecological speciation in *Rediviva* and, *vice versa*, may drive *Diascia* (co-)speciation. Alternatively, only one partner might speciate in response to the other (pollinator or host shift). Hence, cophylogenetic analyses are required to address if diversification between *Rediviva* and *Diascia* is dominated by cospeciation or shift events.

The overall aim of my thesis is thus to understand the evolution of a bizarre morphological adaptation, elongated forelegs, in *Rediviva* bees and how FLL itself impacts the genetic population structure and diversification of *Rediviva*, which in turn might affect diversification in its main host plant *Diascia*. Each of the following three chapters addresses a specific aspect of FLL evolution and thereby contributes to an understanding of the adaptation and evolution of a key pollinator, *Rediviva*, in the Cape biodiversity hotspot.

In **Chapter I** I explore the evolution of elongated forelegs across the whole genus *Rediviva*. I first reconstructed the phylogeny of the genus based on seven genes to infer the number of origins of

elongated forelegs. Moreover, I determined the rate of FLL evolution and tested if the evolution of long forelegs is linked with other ecologically relevant traits and increased speciation rates.

Chapter II of my thesis focuses on the mutual evolutionary history of *Rediviva* and *Diascia* as *Rediviva-Diascia* interactions might have driven not only mutual adaptation (spur length or FLL) but also speciation processes. By using several cophylogenetic approaches to compare the phylogenies of *Rediviva* (from Chapter I) with its *Diascia* host plant, I investigated if cospeciation or pollinator shifts dominate during *Rediviva* and *Diascia* evolution and spur diversification processes.

Finally, **Chapter III** of my thesis concentrates on the genetics of FLL variation in a *Rediviva* species with one of the most extreme FLL documented for the genus: *Rediviva longimanus*. Specifically, I generated restriction site-associated DNA sequencing (RAD-seq) data for *R. longimanus* pools and investigated if FLL variation between pools is coupled with reduced gene flow and thus increased genetic differentiation. Furthermore, I scanned the *R. longimanus* pools for signals of selection to identify candidate genes associated with FLL variation.

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Chapter I

Playing with extremes: origins and evolution of exaggerated female forelegs in South African *Rediviva* bees

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Abstract

Despite close ecological interactions between plants and their pollinators, only some highly specialised pollinators adapt to a specific host plant trait by evolving a bizarre morphology. Here we investigated the evolution of extremely elongated forelegs in females of the South African bee genus *Rediviva* (Hymenoptera: Melittidae), in which long forelegs are hypothesised to be an adaptation for collecting oils from the extended spurs of their *Diascia* host flowers. We first reconstructed the phylogeny of the genus *Rediviva* using seven genes and inferred an origin of *Rediviva* at around 29 MYA (95% HPD= 19.2-40.5), concurrent with the origin and radiation of the Succulent Karoo flora. The common ancestor of *Rediviva* was inferred to be a short-legged species that did not visit *Diascia*. Interestingly, all our analyses strongly supported at least two independent origins of long legs within *Rediviva*. Leg length was not correlated with any variable we tested (ecological specialisation, *Diascia* visitation, geographic distribution, pilosity type) but seems to have evolved very rapidly. Overall, our results indicate that foreleg length is an evolutionary highly labile, rapidly evolving trait that might enable *Rediviva* bees to respond quickly to changing floral resource availability.

Chapter II

Should I stay or should I go? Pollinator shifts dominate the evolutionary history of South African *Rediviva* bees and their *Diascia* host plants

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Abstract

Plant–pollinator interactions are often highly specialised, which may be a consequence of co-evolution. Yet when plants and pollinators co-evolve, it is not clear if this will also result in frequent cospeciation. Here, we investigate the mutual evolutionary history of South African oil-collecting *Rediviva* bees and their *Diascia* host plants, in which the elongated forelegs of female *Rediviva* have been suggested to coevolve with the oil-producing spurs of their Diascia hosts. After controlling for phylogenetic nonindependence, we found *Rediviva* foreleg length to be significantly correlated with *Diascia* spur length, suggestive of co-evolution. However, as trait correlation could also be due to pollinator shifts, we tested if cospeciation or pollinator shifts have dominated the evolution of *Rediviva–Diascia* interactions by analysing phylogenies in a cophylogenetic framework. Distance-based cophylogenetic analyses (PARAFIT, PACo) indicated significant congruence of the two phylogenies under most conditions. Yet, we found that phylogenetic relatedness was correlated with ecological similarity (the spectrum of partners that each taxon interacted with) only for *Diascia* but not for *Rediviva*, suggesting that phylogenetic congruence might be due to phylogenetic tracking by *Diascia* of *Rediviva* rather than strict (reciprocal) co-evolution. Furthermore, event-based reconciliation using a parsimony approach (CORE-PA) on average revealed only 11–13 cospeciation events but 58–80 pollinator shifts. Probabilistic cophylogenetic analyses (COALA) supported this trend (8–29 cospeciations vs. 40 pollinator shifts). Our study suggests that diversification of *Diascia* has been largely driven by *Rediviva* (phylogenetic tracking, pollinator shifts) but not vice versa. Moreover, our data suggest that, even in co-evolving mutualisms, cospeciation events might occur only infrequently.

Chapter III

Small and genetically highly structured populations in a long-legged bee, *Rediviva longimanus*, as inferred by pooled RAD-seq

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Abstract

Adaptation to local host plants may impact a pollinator's population genetic structure by reducing gene flow and driving population genetic differentiation, representing an early stage of ecological speciation. South African *Rediviva longimanus* bees exhibit elongated forelegs, a bizarre adaptation for collecting oil from floral spurs of their *Diascia* hosts. Furthermore, *R. longimanus* foreleg length (FLL) differs significantly among populations, which has been hypothesised to result from selection imposed by inter-population variation in *Diascia* floral spur length. Here, we used a pooled restriction site-associated DNA sequencing (pooled RAD-seq) approach to investigate the population genetic structure of *R. longimanus* and to test if phenotypic differences in FLL translate into increased genetic differentiation (i) between *R. longimanus* populations and (ii) between phenotypes across populations. We also inferred the effects of demographic processes on population genetic structure and tested for genetic markers underpinning local adaptation. Populations showed marked genetic differentiation (average $F_{ST} = 0.165$), though differentiation was not statistically associated with differences between populations in FLL. All populations exhibited very low genetic diversity and were inferred to have gone through recent bottleneck events, suggesting extremely low effective population sizes. Genetic differentiation between samples pooled by leg length (short *versus* long) rather than by population of origin was even higher ($F_{ST} = 0.260$) than between populations, suggesting reduced interbreeding between long and short-legged individuals. Signatures of selection were detected in 1,119 (3.8 %) of a total of 29,721 SNP markers. Populations of *R. longimanus* appear to be small, bottlenecked and isolated. Though we could not detect the effect of local adaptation (FLL in response to floral spurs of host plants) on population genetic differentiation, short and long legged bees appeared to be partially differentiated, suggesting incipient ecological speciation. To test this hypothesis, greater resolution through the use of individual-based whole-genome analyses is now needed to quantify the degree of reproductive isolation between long and short legged bees between and even within populations.

General discussion

In this thesis I investigated the evolutionary genetics of elongated forelegs and foreleg length (FLL) variation in *Rediviva* bees, which was hypothesised to be under strong selection due to host plant spur length and potentially causing *Rediviva* to coevolve with its main host *Diascia*. While former studies concentrated on investigating the morphology of *Rediviva* legs (Kuhlmann & Hollens, 2015), specifically the ecology of the *Rediviva-Diascia* interaction and the trait (FLL- spur length) matching (Hollens, van der Niet, Cozien, & Kuhlmann, 2016), this thesis expands previous research by studying the evolutionary history of *Rediviva-Diascia* interactions and the genetics and evolution of the key trait, FLL, underlying this interactions.

In agreement with the recent literature on the evolution of pollinator-plant interactions (Chacoff, Resasco, & Vázquez, 2017; Russo, Miller, Tooker, Bjornstad, & Shea, 2018; Vázquez et al., 2018), the results of my study presented in Chapter II suggest that diversification processes of *Rediviva* and *Diascia* are not reciprocally linked, i.e. there are only few cospeciation events. However, diversification processes in the plant seem to be affected by its pollinator *Rediviva* as I detected a high frequency of pollinator shift events in cophylogenetic analyses (Chapter II). This is in accord with the presumably high ecological flexibility of *Diascia* plants, as supported by a lack of phylogenetic signal for spur length (data not shown) and suggests that spur length variation is shaped by selection rather than drift. Ancestral state reconstructions also rendered several origins of long spurs more likely than a single origin (data not shown), and thus enabling *Diascia* to frequently shift pollinators during their evolutionary history. Hence, spur length adaptation to *Rediviva* pollinators might have had a strong impact on *Diascia* diversification. Studies on other South African plants also support a role for pollinators and pollinator shifts in plant speciation (Johnson & Steiner, 2003; Johnson, 2010; Pauw, Stofberg, & Waterman, 2009; Valente, Manning, Goldblatt, & Vargas, 2012; van der Niet & Johnson, 2009; van der Niet, Peakall, & Johnson, 2014). For some Coryciinae orchids pollinated by *Rediviva*, it has also been suggested that host-specific pollinarium attachment sites at the bees' body prevent hybridisation between orchid species and thus shifts in pollinarium attachment sites on *Rediviva spp.* (rather than shifts between pollinator species itself) might have played a role in Coryciinae speciation (Waterman et al., 2011).

At the same time, *Rediviva* diversification might be also affected by interactions with *Diascia* not via cospeciation but via host shifts (i.e. 'host-shift speciation', the bee rather than the plant is shifting interaction partner and diversifies), which might also occur at a non-negligible rate. This hypothesis is supported by my cophylogenetic analyses, which also revealed frequent shifts when the plant was treated as host (see Chapter II). In addition, *Rediviva* is ecologically very flexible since any given *Rediviva* interacts with many *Diascia* species (mean host number = 5) than *vice versa* (mean pollinator

number = 2) and FLL, the key trait important for interactions with *Diascia* plants, seems to evolve rapidly and thus allows for switching between host plants. Specifically, I inferred at least two origins of long forelegs, a high evolutionary rate and no phylogenetic signal for FLL, all hinting to the rapid evolution of FLL and thus ecological flexibility (Chapter I). Consequently, although there is only little evidence for cospeciation in our cophylogenetic analyses, *Rediviva* might diversify via shifts in host plant visitation, as also hypothesised for other insects (De Vienne et al., 2013; Doña et al., 2017; Fordyce, 2010).

Furthermore, biogeographic events as well as past and recent climatic conditions might also play a central role for the diversification of *Rediviva* and *Diascia*. First, biogeographic reconstructions for *Rediviva* (Chapter I) and *Diascia* (Cron et al, in prep.) indicated the occurrence of several vicariance events that might have driven diversification. Second, the current climate (high seasonal rainfall, often inclement weather) in the winter rainfall area has been suggested to drive the microevolution of bees (Kahnt, Soro, Kuhlmann, Gerth, & Paxton, 2014; Kuhlmann, 2009), including *Rediviva* (Chapter III), and hence in turn affect *Rediviva* (and *Diascia*) diversification. Third, historical climatic and geological events were also suggested to have contributed to the flourishing of the biodiversity of the entire Cape flora (Cowling et al., 2009; Neumann & Bamford, 2015; Schnitzler et al., 2011).

Although I found little evidence for cospeciation as a major force driving the evolution of the *Rediviva-Diascia* system (Chapter II), this does not preclude coevolution between *Rediviva* and *Diascia* populations. As mentioned before, coevolution is likely to be restricted not only in space (coevolution hotspots in a geographic mosaic of coevolution) but also in time (coevolutionary interludes across evolutionary history) and might thus not translate into macroevolutionary effects, i.e. cospeciation of interacting species (Janz, 2011). Furthermore, as most *Rediviva* and *Diascia* species usually have multiple interaction partners across their whole distribution range and a bee's FLL seems to be correlated with the spur length of the whole host plant community rather than an individual host species (Hollens et al., unpublished), *Rediviva* and *Diascia* might exhibit diffuse rather than pairwise coevolution. Hence, there is probably only little potential for close evolutionary coupling, i.e. cospeciation, between these two taxa even if they are diffusely coevolving.

The most likely scenario is probably a mixture of pollinator shifts and diffuse coevolution in a geographic mosaic, with only some populations coevolving. In order to test if *Rediviva* and *Diascia* populations show diffuse coevolution and a geographic mosaic of coevolution, future studies should investigate *Rediviva- Diascia* interactions and the key traits involved in theses interactions, FLL and spur length, across the whole distribution range of *Rediviva* and *Diascia*. Such studies should start by testing for a correlation of FLL and spur length across multiple populations for several interacting *Rediviva* and *Diascia* species and thereafter investigate the fitness consequences of trait matching and

mismatching. Trait correlation analyses have already been carried out at the species-level while taking into account phylogenetic non-independence (Chapter II; Pauw et al., 2017) and for a few population pairs in the winter (Hollens et al., 2016; Hollens et al., unpublished) and summer rainfall area (Steiner & Whitehead, 1990, 1991). They all suggested that there is indeed a correlation between FLL and spur length. However, none of these studies investigated if *Rediviva* and *Diascia* reciprocally exert selection on each other's traits, FLL and spur length. Moreover, it is unknown if trait matching is associated with higher fitness in both, *Rediviva* and *Diascia* (e.g. amount of oil gained in the case of the bee and seed set in the case of the plant) than a trait mismatch. Such knowledge is nonetheless essential to support a coevolutionary relationship between *Rediviva* and *Diascia*.

Rediviva FLL, the key trait involved in interactions with the host plant, might also affect the micro- and macroevolution of *Rediviva* itself. For FLL to influence *Rediviva* speciation it requires FLL to be under divergent selection and to affect reproduction and reproductive isolation. The results of my thesis and other studies (Hollens et al., 2016; Pauw et al., 2017, Hollens et al. unpublished) argue for non-negligible selection pressures on FLL, although they do not allow conclusions about the form of selection, i.e. divergent or balancing. First, FLL is likely to be under selection as it may have great fitness consequences (Hollens et al., 2016; Pauw et al., 2017). Second, the lack of a phylogenetic signal for FLL suggests that FLL is not strongly determined by phylogenetic ancestry. In addition, models including the effect of selection (Ornstein-Uhlenbeck) better explain FLL variation than pure Brownian motion models that only account for drift (Chapter I). Hence, it is unlikely that FLL variation is solely driven by drift but rather by strong selection. I thus expected the loci underlying FLL to appear as F_{ST} outlier in the genome-wide distribution of RAD tags that I used to compare populations of *Rediviva longimanus* in Chapter III. Indeed, I was able to identify some potential candidate genes regulating FLL in *R. longimanus*, including the Hox gene *Sex combs reduced* (*Scr*), despite a potentially high impact of gene flow (Chapter III).

FLL, like most fitness-relevant traits (Storz, 2005), is probably a quantitative trait and thus controlled by many interacting loci. In general, the size of insect body parts is thought to be controlled mainly via cell number and, probably to an lesser extent, cell size (Nijhout, 2003). Genetic or environmentally induced (plastic) changes in the rate or duration of cell proliferation might be the primary mechanisms by which an increase in cell number and thus the evolution of enlarged structures is achieved (Emlen & Allen, 2003). Genes known to affect the rate of cell proliferation within imaginal discs of insect larvae, which might play a role for FLL regulation as well, are the morphogens *wingless* (w*g*) and *decapentaplegic* (*dpp*), *Distal-less* (*Dll*), *Notch* and *Epidermal growth factor* (*EFG*) (Emlen & Allen, 2003). Specifically, changes in the shape and intensity of morphogen gradients seems to be an important mechanism by which *Hox* genes are able to modulate organ and appendage growth (Crickmore & Mann, 2010). For example, altered interactions between *dpp* with the Hox gene

UbdA/Ubx seem to be responsible for exaggerated insect structures, including elongated hindlegs of grasshoppers and crickets, exaggerated beetle horns (Lavine, Gotoh, Brent, Dworkin, & Emlen, 2015) or wing reduction to halters in dipterans (Crickmore & Mann, 2010). I hypothesise that in *Rediviva, dpp* might also possibly interact with specific Hox genes to regulate leg development and the elongation of forelegs.

Genes involved in leg development have been identified in several insects, including honey bees (Khila, Abouheif, & Rowe, 2009; Mahfooz, Li, & Popadić, 2004; Mahfooz, Turchyn, Mihajlovic, Hrycaj, & Popadić, 2007; Refki et al., 2014; Refki & Khila, 2015; Struhl, 1982; Walldorf et al., 2010). These genes include *Ultrabithorax* (*Ubx*), *Sex combs reduced* (*Scr*) and *Antennapedia* (*Antp*), all of which belong to the Hox gene family. Among the three Hox genes mentioned, *Scr* is probably the most promising candidate for FLL regulation in *Rediviva* since it is generally expressed in the first pair of legs (Walldorf et al., 2010; Rogers, Peterson, & Kaufman, 1997; Struhl, 1982), while the other two genes are only known to be expressed in the mid- and hind legs (Mahfooz et al., 2004, 2007; Refki et al., 2014; Stern, 2003). Indeed, *Scr* was also among the candidate genes I inferred to be under strong selection in *R. longimanus* (Chapter III). Interestingly, *Scr* is also known to show sex specific differences in expression levels, leading to leg combs in *Drosophila* males (Barmina & Kopp, 2007). Thus, I suggest that *Scr* may be also a central component in the sex specific expression of extended forelegs in *Rediviva*.

In *Drosophila*, sex is determined by the ratio of sex chromosomes to autosomes which triggers a genetic cascade via the master regulator gene *Sex-lethal* (*Sxl*), leading to sexual differentiation and dimorphism in e.g. body size (Horabin, 2005). *Sxl* regulates the splicing of the RNA-binding factor *transformer* (*tra*) such that only females will have a functional Tra protein, which in turn induces the splicing of the *doublesex* (*dsx*) transcription factor into a female specific isoform (*dsxF*) while the male isoform (*dsxM*) is produced by default (Kopp, 2012). The different *dsx* isoforms then trigger the development of sex specific characters and, in the first pair of legs, *dsx* and *Scr* enhance each other's expression to produce sex combs in males (Kopp, 2012; Tanaka, Barmina, Sanders, Arbeitman, & Kopp, 2011).

Moreover, temporal and spatial modifications of *dsx* gene expression has been suggested to play a major role for the evolution of many sexually dimorphic insect traits and trait exaggerations (Tanaka et al., 2011). *dsx* is known to be important for sex-specific trait growth not only in *Drosophila* but also in other dipterans, various beetle taxa and ants (Klein et al., 2016; Lavine et al., 2015) and acts via binding to the *cis*-regulatory region of downstream targets. For example, in several beetle species *dsx* regulates the expression of exaggerated horns and mandibles in the males (Lavine et al., 2015).

In bees, sex is not determined by the ratio of autosomes to sex chromosomes but rather by the zygosity of the sex determining locus (*complementary sex determination*, *csd,* locus in *Apis* and *feminizer*, *fem,* in many non-*Apis* bees; Charlesworth & Mank 2010). The bees' *csd* locus probably encodes a protein (SR) that is homologous to *tra* and involved in splicing of various mRNAs, including a *dsx* homolog (Cristino, Nascimento, Costa, & Simões, 2006), resulting in four sex specific *dsx* isoforms in bees (Cho, Huang, & Zhang, 2007; Siegal & Baker, 2005). Hence, a pathway similar to *Drosophila* that involves the interaction between *dsx* and *Scr* might regulate sexual dimorphism in *Rediviva*, with female bees possessing not only slightly larger body sizes but also massively larger forelegs than their male congeners (Whitehead, & Steiner, 2001; Whitehead, Steiner, & Eardley, 2008).

In addition to genetic factors, environmentally induced phenotypic plasticity and epigenetic regulation of gene expression might also be relevant for FLL regulation. Plasticity of FLL could involve (i) insulin-triggered growth factors, which control the rate of cell proliferation of entire imaginal disc, or (ii) the developmental hormones Juvenile hormone (JH) and Ecdysone*,* that regulate the duration of growth (Emlen & Allen, 2003), or (iii) an interaction of those mechanisms (Mirth et al., 2014). However, the insulin-signalling cascade, which is regulated via variation in nutrition, is unlikely to explain elongated forelegs in *Rediviva* as bee larvae developing longer legs are likely not fed with more oil than short-legged ones. Hormonal control is thus a more likely alternative for plastic responses in FLL. For example, it is known that *dsx* may sex specifically modulate the sensitivity of organs to the hormone JH, resulting in exaggerated mandibles in male stag beetles (Gotoh et al., 2014). Similarly, plastic responses of FLL in female *Rediviva* may be possible via the interaction between *JH* and *dsx*. Furthermore, since most sexually dimorphic traits, including *dsx*-regulated sexual dimorphisms (Tanaka et al., 2011; see above), are due to differences in gene expression (Ellegren & Parsch, 2007) genetic elements such as microRNAs or epigenetic regulation via GC-methylation or histone acetylation might be involved in regulating the expression of genes determining FLL.

Future studies to address the genetic underpinning of FLL variation in *Rediviva* should thus sequence our most promising candidate gene *Scr* in individuals of different populations (and species) to confirm the fixation of different SNPs in populations with different FLL and thereby better support a role of *Scr* in FLL regulation. Furthermore, since FLL is probably controlled by several genes and I investigated only a small proportion of the genome with a reduced genome representation sequencing approach (RAD-Seq, Chapter III), I probably have missed important genes involved in FLL regulation such as *dsx*. Hence, whole genome sequencing of populations, pooled or individually barcoded, that exhibit marked differences in FLL are required to fully address the genetic architecture of FLL regulation. In addition, real-time PCR amplification or transcript abundance estimation from EST sequencing of cDNA libraries prepared from imaginal discs of *Rediviva* larvae from both sexes are needed to give critical insights into potential differences in gene expression and the genetic

architecture of FLL. However, given the difficulties of excavating *Rediviva* nest from the soil and the lack of established procedures to rear *Rediviva* in the lab (Kuhlmann, pers. communication), such studies are unlikely to be possible in the near future.

Moreover, in order to substantiate a role of FLL in *Rediviva* diversification, FLL also needs to be linked to reproductive isolation. As pointed out in Chapter III, divergent mate preference due to varying FLL is unlikely as males are not usually the choosy sex in bees nor do they vary in FLL, excluding the possibility of assortative mating. Reproductive isolation due to habitat/resource preference (plants with fitting spur lengths) is possible (see Chapter III). Furthermore, reproductive isolation might result from a lower viability of immigrant bees with non-matching FLL. Alternatively, post-mating isolation due to intrinsic genetic incompatibilities or (sexually or ecologically dependent) selection against hybrid offspring (Nosil, 2012; Rundle & Nosil, 2005) might occur. Although local *Diascia* communities do not always consist of plants with only extreme spur lengths (short or long) but they contain a mixture of plants showing intra-and interspecific variation in spur lengths, there nevertheless seems to be a significant difference in the mean community spur length between sites (Hollens et al. unpublished). Therefore reproductive isolation caused by habitat isolation or a reduced fitness of immigrants or hybrids due to non-optimal phenotypes, FLL in particular, might indeed be possible. However, the FLL of a female bee does not have to perfectly match spur length in order for it to extract oil (Hollens et al. 2016). Moreover, the composition of *Diascia* communities and spur lengths might vary between years (Kuhlmann, pers. communication). Thus, FLL might not be under strong directional but rather balancing selection. Hence, none of these mechanisms proposed might be strong enough to lead to reproductive isolation, if FLL is indeed under balancing selection.

Independently of the mechanism driving potential reproductive isolation due to FLL, the loci regulating FLL need to be coupled with the loci effecting reproductive isolation. Such coupling could be due to pleiotropic effects of loci underlying FLL or due to linkage between the loci regulating FLL and those causing reproductive isolation (Nosil, Harmon, & Seehausen, 2009). The four-phase-model of speciation-with-gene-flow by Feder et al. (2012) assumes that initially (phase 1) only a few loci under strong divergent selection are differentiated between populations (Fig. 1). In the following phases, differentiation sequentially builds up; first only in the regions surrounding the loci under strong selection (divergence hitchhiking, phase 2) but, later on, genome-wide as new mutations establish due to selection and drift (genome hitchhiking, phase 3) and the baseline level of divergence between populations increases. The final phase (phase 4) is reached when there is no or only low gene flow across the genomes of the two taxa. Such a model might also apply to *Rediviva* populations that vary in FLL, if FLL indeed experiences strong divergent selection.
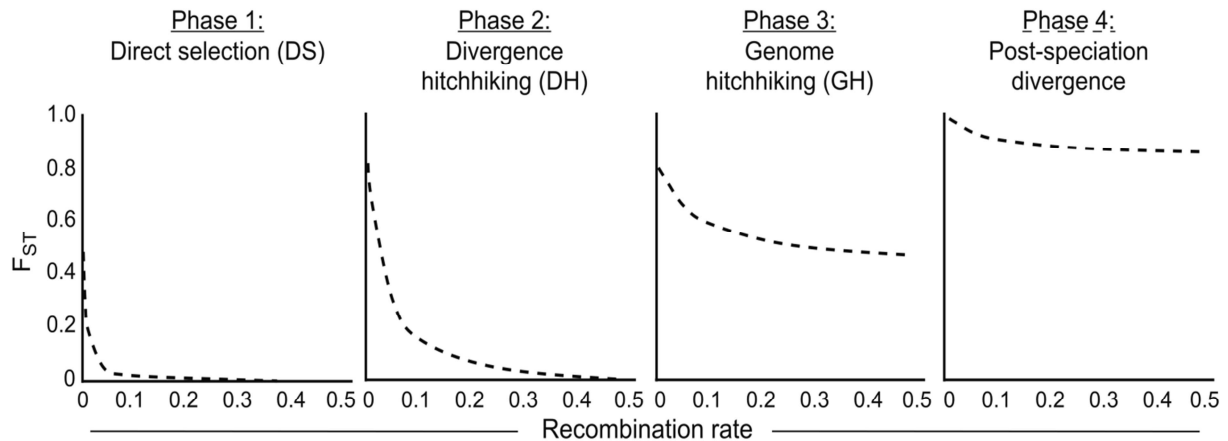
Fig. 1 The four-phase-model of speciation-with-gene-flow by Feder et al. (2012).

Despite FLL being an important ecological adaption with potentially great evolutionary flexibility, my studies did not strongly support FLL as a driver of *Rediviva* microevolution and diversification processes. I neither found a correlation between long forelegs and diversification rates across the whole genus (Chapter I) nor was genetic differentiation of *Rediviva longimanus* populations related to inter-population (intraspecific) variation in FLL (Chapter III). Nevertheless, the latter results might also be due to a lack of statistical power, since I investigated only few populations (N=4). Additionally, I detected pronounced genetic differentiation between sequencing pools, pooled according to FLL (Chapter III), hinting to a role of FLL in microevolution. Hence, in order to rule out a role of FLL in *Rediviva* microevolution, further studies are needed to investigate gene flow and reproductive isolation between multiple populations of (ideally multiple) *Rediviva* species that show pronounced intraspecific and inter-population variation in FLL.

My population genomic analyses of *R. longimanus* (Chapter III) also suggested that the bees are of conservation concern since populations seem to be small, scattered and highly genetically differentiated. Furthermore, *R. longimanus* populations exhibited low genetic diversity and might experience population bottlenecks (Chapter III); this is potentially a result of the inclement weather during the main flowering season that might markedly reduce activity and gene flow between bee populations. Hence, reduced genetic diversity and high population genetic differentiation might be a general feature of bees in the winter-rainfall area and thus, many bee species, including *Rediviva*, might actually suffer from an increased extinction risk. If this is indeed the case, *Rediviva* bees should be given conservation priority in the Greater Cape Floristic Region (GCFR) biodiversity hotspot as they are endemic to South Africa and Lesotho.

This thesis is the first to provide insights into the evolution of an important pollinator, *Rediviva,* in the GCFR biodiversity hotspot and into the population genomics of one of the most abundant *Rediviva*

species, in relation to the evolution of FLL, the key adaptation to its host plant *Diascia*. Specifically, this thesis investigated if and how the adaptation of *Rediviva* bees to their *Diascia* host plants affects *Rediviva* micro- and macroevolution, which in turn may affect *Diascia* diversification. Overall, my results suggest that FLL is a rapidly evolving trait, potentially under strong selection. Although my studies suggest that *Rediviva* pollinator shifts drive *Diascia* diversification, I did not find strong evidence that the interactions with *Diascia* and FLL adapation affect the micro- and macroevolution of *Rediviva*. Future studies are now required to address the interplay between coevolution and shifts at the population level as well as potential reproductive isolation due to FLL and the complete genetic architecture of FLL variation. The results of my thesis provide the fundaments for such studies and by taking an evolutionary perspective contribute to the understanding and protection of a central component of the biodiversity in the GCFR biodiversity hotspot. Since as Dobzhansky (1973) said: 'Nothing in biology makes sense except in the light of evolution.'

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Acknowledgements

A. Curriculum vitae

Personal details	
Name:	Belinda Kahnt
Email:	belinda-k@gmx.de
Date and location of birth:	6 July 1987, Borna, Germany

Education	
Since 12/2013	**PhD studies** *Martin-Luther-University Halle-Wittenberg* (Germany) Institute of Biology/ General Zoology *Dissertation title:* ‚Evolutionary genetics of a bizarre adaptation: giant front legs of Rediviva bees as an adaptation to their *Diascia* hosts in the winter rainfall area of South-Africa'
09/2012	**Master's degree** (grade: 1.1) *Master thesis*: 'Population genetics of a rare bee in the Succulent Karoo vegetation region of South Africa'
09/2010	**Bachelor's degree** (grade: 1.3; with honours) *Bachelor thesis*: 'Importance of the range size and biogeographical distribution for evaluating the national responsibility for reptiles in Europe'
10/2007 - 09/2012	Study of biology (B.Sc. and M.Sc.) Specialisation: *'Biodiversity and evolution of animals'* *University of Leipzig* (Germany)

Conferences	
08/2019	European Society for Evolutionary Biology (ESEB) conference, Turku (Finland), talk: Should I stay or should I go? Pollinator shifts dominate the evolutionary history of South African Rediviva bees and their Diascia host plants
03/2019	DGaaE-Entomology conference, Halle (Germany), talk: Not the perfect match? Pollinator shifts rather than cospeciation dominate the evolutionary history of Rediviva bees and their Diascia host plants
12/2018	*Annual yDiv conference*, Leipzig (Germany), talk: Not the perfect match? Pollinator shifts rather than cospeciation dominate the evolutionary history of *Rediviva* bees and their *Diascia* host plants
09/2018	*EurBee* conference, Ghent (Belgium), talk: Not the perfect match? Biogeography and pollinator shifts rather than cospeciation dominate the evolutionary history of *Rediviva* bees and their *Diascia* host plants in the Cape biodiversity hotspot
12/2017	*Annual yDiv conference*, Leipzig (Germany), talk: Cabinet of curiosities: the evolution of exaggerated forelegs in South African *Rediviva* bees

08/2017	*European Society for Evolutionary Biology (ESEB)* conference, Groningen (The Netherlands), poster: Cabinet of curiosities: the evolution of exaggerated forelegs in South African *Rediviva* bees
12/2016	*British Ecological Society (BES)* conference, Liverpool (UK), talk: Pollinator evolution and adaptation in South Africa: phylogenetics and co-phylogenetics of the bizarre *Rediviva* buzzer bee and its associated host plants
12/2016	*Annual yDiv conference*, Leipzig (Germany), talk: Phylogenetics and cophylogenetics of *Rediviva* bees and its associated host plants
11/2016	*Phylogenetic symposium: Evolution meets Ecology*, Leipzig (Germany)
08/2015	*European Society for Evolutionary Biology (ESEB)* conference, Lausanne (Switzerland), poster: Phylogenetics and evolution of extremely elongated front legs in the endemic South African bee genus *Rediviva*
10/2014	*Hymenopterologen-Tagung*, Stuttgart (Germany), talk: Insights into the phylogeny of the endemic oil-collecting bee genus *Rediviva* based on nuclear and mitochondrial data
10/2014	*yDiv symposium*, Leipzig (Germany), talk: Evolutionary genetics of a bizarre adaptation in the South African bee genus *Rediviva*
09/2014	*iSEQ symposium on methods and applications of next-generation sequencing in evolutionary research*, Leipzig (Germany)
09/2012	*EurBee* conference, Halle (Germany), poster: Population genetics of the rare halictid bee *Patellapis doleritica* of the Succulent Karoo biome of South Africa

Research stays abroad

09/2016	Cophylogenetic analyses, **University of the Witwatersrand**, Johannesburg (South Africa)
04/2015-05/2015	Phylogenetic analyses, **Cornell University**, Ithaca (USA)

Grants and awards

09/2018	Price for the **best talk** at the *Eurbee* 2018
04/2016	**Travel grant** from the *Studienstiftung des deutschen Volkes* for the research stay at the University of the Witwatersrand (South Africa)
12/2014	**Travel grant** from *iDiv* for the research stay at the Cornell University (USA)
03/2014	**Research grant** from *iDiv* for Next-Gen sequencing (*Rediviva* phylogeny)
12/2013-11/2016	**Scholarship** of the *Studienstiftung des deutschen Volkes*

B. Publication list

Kahnt , B., Hattingh, W. N., Theodorou, P., Wieseke, N., Kuhlmann, M., Glennon, K. L., van der Niet, T., Paxton,R. J., Cron, G. V. (2019) Should I stay or should I go? Pollinator shifts dominate the evolutionary history of South African *Rediviva* bees and their *Diascia* host plants. *Molecular Ecology* 28: 4181-4133. doi: 0.1111/mec.15154

Kahnt, B., Theodorou, P., Soro, A., Hollens-Kuhr, H., Kuhlmann, M., Pauw, A., Paxton Paxton. R. J. (2018) Small and genetically highly structured populations in a long-legged bee, *Rediviva longimanus*, as inferred by pooled RAD-seq. *BMC Evolutionary Biology* 18: 196. doi: 10.1186/s12862-018-1313-z

Theodorou, P., Radzevičiūte, R., **Kahnt, B.**, Soro, A., Grosse, I., Paxton. R. J. (2018) Genome-wide single nucleotide scan suggests adaptation to urbanization in an important pollinator, the red-tailed bumblebee (*Bombus lapidarius* L.). *Proceedings of the Royal Society B* 285: 20172806. doi: 10.1098/rspb.2017.2806

Kahnt, B., Montgomery, G. A., Murray, E., Kuhlmann, M., Pauw, A., Michez, D., Paxton, R. J., Danforth, B. N. (2017) Playing with extremes: origins and evolution of exaggerated female forelegs in South African *Rediviva* bees. *Molecular Phylogenetics and Evolution* 115: 95-105. doi: 10.1016/j.ympev.2017.07.025

Pauw, A., **Kahnt, B**., Kuhlmann, M., Michez, D., Montgomery, G. A., Murray, E., & Danforth, B. N. (2017) Long-legged bees make adaptive leaps: linking adaptation to coevolution in a plant–pollinator network. *Proceedings of the Royal Society B* 284: 20171707. doi: 10.1098/rspb.2017.1707

Kahnt, B., Gerth, M., Paxton, R. J., Bleidorn, C., Husemann, M. (2015) The complete mitochondrial genome of the endemic and highly specialized South African bee species *Rediviva intermixta* (Hymenoptera: Melittidae), with a comparison with other bee mitogenomes. *Biological Journal of the Linnean Society* 116: 940-953. doi: 10.1111/bij.12627

Kahnt, B., Soro, A., Kuhlmann, M., Gerth, M., Paxton, R. J. (2014) Insights into the biodiversity of the Succulent Karoo hotspot of South Africa: the population genetics of a rare and endemic halictid bee, *Patellapis doleritica. Conservation Genetics* 15: 1491-1502. doi: 10.1007/s10592-014-0633-9

C. Declaration of own contribution to the original articles presented in this thesis

I. **Kahnt, B**., Montgomery, G. A., Murray, E., Kuhlmann, M., Pauw, A., Michez, D., Paxton, R. J., Danforth, B. N. (2017) Playing with extremes: origins and evolution of exaggerated female forelegs in South African *Rediviva* bees. *Molecular Phylogenetics and Evolution* 115: 95-105. doi: 10.1016/j.ympev.2017.07.025

Design of the project: 50%
Collection of field data: 0%
Experimental work: 50%
Data analysis: 80%
Wrote the paper: 80%

II. **Kahnt, B**., Hattingh, W. N., Theodorou, P., Wieseke, N., Kuhlmann, M., Glennon, K. L., van der Niet, T., Paxton, R. J., Cron, G. V. (2018) Should I stay or should I go? Pollinator shifts dominate the evolutionary history of South African *Rediviva* bees and their *Diascia* host plants. *Molecular Ecology* 28: 4181-4133. doi: 0.1111/mec.15154

Design of the project: 100%
Data analysis: 100%
Wrote the paper: 80%

III. **Kahnt, B**., Theodorou, P., Soro, A., Hollens-Kuhr, H., Kuhlmann, M., Pauw, A., Paxton Paxton. R. J. (2018) Small and genetically highly structured populations in a long-legged bee, *Rediviva longimanus*, as inferred by pooled RAD-seq. *BMC Evolutionary Biology* 18: 196. doi: 10.1186/s12862-018-1313-z

Design of the project: 80%
Collection of field data: 0%
Experimental work: 100%
Data analysis: 90%
Wrote the paper: 80%

D. Eidesstattliche Erklärung

Halle, den 08.03.2019

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder bei der Naturwissenschaftlichen Fakultät I der Martin-Luther-Universität Halle-Wittenberg, noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre, dass ich mich bisher noch nicht um den Doktorgrad beworben habe.

Ferner erkläre ich an Eides statt, dass ich dies Arbeit selbstständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Belinda Kahnt