

Leg regeneration stunts wing growth and hinders flight performance in a stick insect (*Sipyloidea sipyilus*)

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Major morphological structures are sometimes produced not once, but twice. For example, stick insects routinely shed legs to escape a predator or tangled moult, and these legs are subsequently re-grown. Here, I show that in *Sipyloidea sipyilus*, re-growth of a leg during development causes adults to have disproportionately smaller wings and increases wing loading. These morphological consequences of leg regeneration led to significant reductions in several biologically relevant measures of individual flight performance. This previously unrecognized tradeoff between legs and wings reveals the integrated nature of phasmid phenotypes, and I propose how this tradeoff may have shaped phasmid evolution.

Keywords: tradeoffs; regeneration; stick insects; evolution; flight

1. INTRODUCTION

Tradeoffs are fundamental to life history and evolution, since they can link the expression of multiple traits, impeding the independent evolution of either trait (Needham & Stearns 1992; Roff 1992). One intuitive way to study tradeoffs is to quantify the costs of allocating resources to the production of a focal trait; although it is obvious that developing structures require energy and resources to grow, it is generally not clear just how expensive this investment is to the animal itself. In fact, these 'production costs' of trait development can be surprisingly difficult to quantify. For this reason, many of the best estimates to date involve unusually large or conspicuous traits (e.g. the exaggerated ornaments and weapons of sexual selection), presumably because they are accompanied with correspondingly large, and therefore measurable, costs (Møller 1989; Nijhout & Emlen 1998; Emlen 2001). In this paper, I demonstrate a powerful alternative approach for studying allocation tradeoffs that capitalizes on the fact that some animals produce a major morphological structure not once, but twice.

A variety of animal taxa shed and regenerate appendages. Many amphibians (Scadding 1980), reptiles (Arnold 1984; Bellairs & Bryant 1985), fishes (Wagner & Misof 1992) and arthropods (Needham 1953; Needham 1965; Bulliere & Bulliere 1985; Vollrath 1990) have the ability to regenerate lost legs or tails, and these appendages can comprise up to 40% of an individual's biomass. Since appendage regeneration requires the allocation of resources, this process can have profound consequences. In some lizards, for example, regeneration of a lost tail

significantly reduced fecundity (Smyth 1974; Maiorana 1977; Dial & Fitzpatrick 1981; Norman & Jones 1993). However, tradeoffs between somatic and reproductive growth are not the only types of allocation tradeoffs that can affect fitness. Here I show for a species of stick insect, *Sipyloidea sipyilus* (Westwood, figure 1), that producing a leg for a second time can affect animal shape, and that this negatively impacts animal performance.

2. STUDY SYSTEM

Stick insects regularly shed and regenerate lost legs. Legs can be lost to encounters with predators; if a predator grasps a leg instead of the body, the leg may be shed and the animal can drop to the ground to escape (Bordage 1905; Carlberg 1986; Brock 1999). Legs can also be lost to complications with moulting. Stick insects moult upside down and separate themselves from their old exoskeleton with elaborate twisting and turning manoeuvres. During moulting, the long and thin legs so effective at conferring crypsis can become entangled in the old cuticle and must be shed to survive (Brock 1999; Maginnis 2000, personal observation). After a leg is lost to a fouled moult or predation event, it is regenerated (immature stick insects always commence regeneration). Complete regeneration of a leg requires three consecutive moults, each producing a successively larger version of the replacement leg (Bordage 1905; Ramme 1931; Wilbert 1953; Carlberg 1992).

Sipyloidea sipyilus, the pink-winged stick insect, lives in the canopies of tropical forests in Madagascar and Southeast Asia (Brock 1999). Like approximately half of all extant phasmid species, *S. sipyilus* has wings (Whiting *et al.* 2003), which they use primarily to control the speed and trajectory of free-fall descents from tree canopies (Carlberg 1984; Maginnis 2000, personal observation; Dudley 2004, personal communication). Free-falls can be self-initiated to facilitate foraging and mate location, or forced by a predation attempt or strong wind. Gliding allows an individual to slow its descent and land on the

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Figure 1. *Sipyloidea sipyilus*, the pink-winged stick insect.

ground without injury, and a combination of gliding and active flapping permits an individual to land on vegetation well above the ground or even to return to the trunk of the same tree (Yanoviak *et al.* 2005; Dudley personal communication). Slow and controlled descents between adjacent tree canopies can prevent a conspicuous landing on the forest floor and/or an energetically costly return to the canopy. In this study, I aimed to determine

(i) the effects of leg regeneration on wing growth and (ii) the consequences of this allocation tradeoff to flight performance.

3. MATERIAL AND METHODS

(a) *Morphological consequences of leg regeneration*

Eggs of *S. sipyilus* were obtained from commercial breeders and reared in growth chambers (day=12 : 12 L : D cycle, 26 : 24 °C). On emergence, 50 nymphs were randomly assigned to one of two treatment groups (zero or one leg regenerated). ‘Regenerated’ animals were induced to regenerate their right hind leg at the beginning of the second instar (eight instars=total development); clasping a limb with forceps causes the animal to drop the leg and commence the regeneration process. Individuals were kept in mesh cages and fed *ad libitum* (bramble, *Rubus idaeus*). One animal died and 14 animals lost additional legs from moulting complications (14/50=28%), so 15 animals were not considered in subsequent analyses. Upon completion of the flight experiments (see later), animals were euthanized and measured for body size (length) and wing area using a stereo-microscope equipped with a digital camera and SCION IMAGE software. Relative wing sizes were compared for the two treatment groups using analysis of covariance (ANCOVA; wing area as the dependent variable; body length as the covariate). Wing loadings (wing area/body size) and relative wing sizes were compared for the two treatment groups using analysis of variance (ANOVA).

(b) *Consequences of leg regeneration on flight performance*

In order to test the hypothesis that stunted wings negatively affect flight performance, free-fall descents were simulated in the laboratory. Within 5 days of eclosion, individuals were released from a 12 foot ladder (height of release was 4.57 m (15 feet) off of the ground). Each individual was released five separate times, and two aspects of every descent were recorded by a second observer: time aloft and type of landing. Time aloft reflected how efficiently individuals used their wings to slow and control their free-fall descent by actively gliding and/or flapping, and impact of landing represented an additional index of descent control (see electronic supplementary material). Individuals in-control of their descent land dorsal side up (i.e. a ‘safe’ landing), and individuals out-of-control land ventral side up or on their side (i.e. a ‘crash’ landing). The average time aloft and the proportion of crash landings were compared for the treatment groups using unpaired *t*-tests assuming unequal variances. The relationship between average time aloft and proportion of crash landings was examined using a correlation test (*r*).

4. RESULTS

(a) *Morphological consequences of leg regeneration*

Leg regeneration stunted the growth of wings. Individuals regenerating a right hind leg during development experienced an approximately 15% decrease in wing area compared with control animals that did not regenerate any legs (1-Factor ANCOVA, $F_{2,35}=29.916$; $p<0.0001$, figure 2a). This regeneration-induced decrease in wing size translated into increased wing loading (1-Factor ANOVA, $F_{2,35}=73.306$; $p<0.0001$, figure 2b).

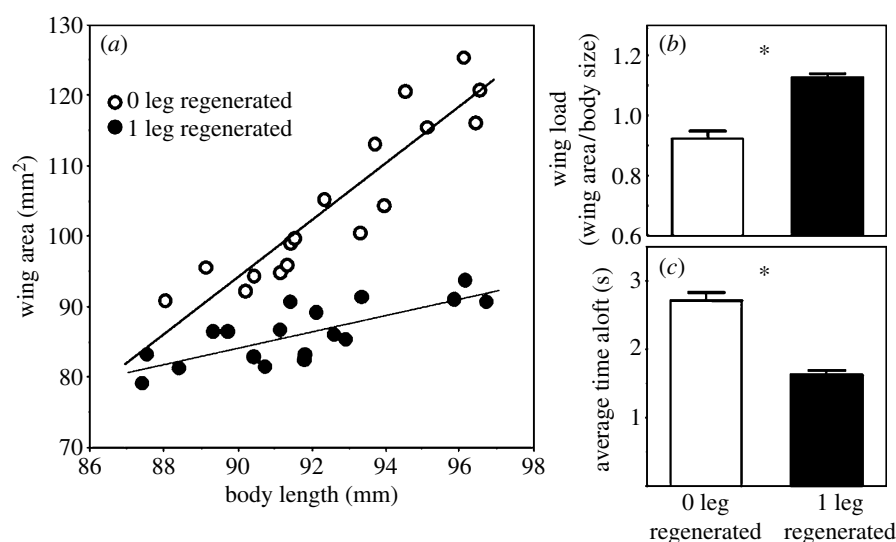


Figure 2. Leg regeneration stunted wing size, increased wing loading and impaired flight performance. Nymphs that regenerated a right hind leg develop into adults with proportionately smaller wings than nymphs not regenerating a leg ((a)1-Factor ANCOVA, $F_{2,35}=29.916$; $p<0.0001$), and this translated into a significant increase in the wing loading of these individuals ((b)1-Factor ANOVA, $F_{2,35}=73.306$; $p<0.0001$). In addition, treatment animals fell faster during free-fall descents than control animals ((c) $t=6.909$; $p<0.0001$); individuals regenerating a leg during development descended at an average rate of 1.6 s (s.e. ± 0.07); control individuals descended at an average rate of 2.7 s (s.e. ± 0.13).

(b) Consequences of leg regeneration on flight performance

In repeated trials, adults which had regenerated a leg during development consistently fell faster than individuals that had not regenerated a leg ($t=6.909$; $p<0.0001$; figure 2c), and a higher proportion of these individuals experienced ‘crash’ landings (45.2% compared to only 11.8% of control animals). The probability of a ‘crash’ landing was correlated with time aloft ($r=-0.644$; $p<0.0001$), suggesting that these measures reliably reflect individual differences in flight performance. Thus, leg regeneration caused individuals to fall faster and crash more frequently—two ecologically relevant metrics of flight performance.

5. DISCUSSION

Only rarely it is possible to appreciate, much less quantify, the costs associated with the growth of morphological traits. Here, by capitalizing on the propensity of stick insects to shed and regenerate appendages, I quantified the costs associated with the growth of a leg. Since individuals can be induced to grow a leg a second time, it is possible to compare the morphology and performance of otherwise similar individuals that differ only in the number of legs that they have produced (six in control individuals versus seven in treatment individuals). In *S. sipylus*, the regeneration of a right-hind leg led to a reduction in wing area. This morphological consequence of leg regeneration translated into decreased flight performance. These results clearly demonstrate that growth of morphological structures in stick insects can be costly, providing empirical support for one of the fundamental tenets of life history.

Rates of leg regeneration are non-trivial in natural populations. I studied a field population of a winged Australian species (*Didymuria violescens*), and found that approximately 25% of adults regenerated at least one leg during development (24.2% of females ($n=411$); 25.6%

of males ($n=392$); Maginnis in preparation). High rates of regeneration in the wild suggest that the tradeoffs resulting from leg re-growth may be pervasive and important agents of selection on stick insect populations (especially in those habitats where vegetation and/or canopy height influence flight behaviour). Consideration of these tradeoffs, as well as how often they are likely to occur, may help explain macro-evolutionary patterns of morphological evolution in stick insects.

Recent phylogenetic studies of phasmatodea showed multiple losses (and potentially regains) of wings during their evolution (Whiting *et al.* 2003; Trueman *et al.* 2004; Whiting & Whiting 2004). Whiting *et al.* (2003) suggested two factors that could have influenced wing evolution: tradeoffs between wings and fecundity and tradeoffs between wings and effective crypsis. In some hemimetabolous insects, wingless females have higher fecundity than winged females (Mole & Zera 1993; Mole & Zera 1994; Zera & Harshman 2001), and stick insects without wings may be more cryptic (Whiting *et al.* 2003). My study suggests an additional and previously unconsidered explanation for an evolutionary loss of wings: resource allocation tradeoffs between wing development and leg regeneration.

Insect wings and wing musculature are notoriously expensive structures to build and maintain (Mole & Zera 1993; Mole & Zera 1994; Zera & Harshman 2001). In addition, the benefits of stick insect wings are not particularly large to begin with: phasmid wings are relatively small for their body sizes, and these insects are relatively weak fliers. Individuals forced to regenerate a leg develop even smaller wings, and their flight performance is significantly worse than those of non-regenerating individuals. This raises the intriguing possibility that wing production in regenerating animals may no longer be cost effective. If true, then populations or habitats with high rates of leg loss (either through predation or complications with moulting) may select for the suppression of wing development all together.

Resource allocation tradeoffs between two very different processes, leg regeneration and wing development, influence the final shape and flight performance of stick insects: they link the development and evolution of legs and wings. Recognizing the integrated nature of phasmid phenotypes may help to explain evolutionary patterns, and offers new insight into the way we perceive the evolution of both legs and wings. More research into other regeneration-induced tradeoffs in other phasmid traits (e.g. fecundity) and morphologies (e.g. wingless species) promises to bring studies of this phenomenon full circle. In particular, experiments that continue to address the performance consequences of allocation tradeoffs provide a context to address if and how these tradeoffs have interacted with their ecology to influence their evolution. This study documents a novel consequence of regeneration, and demonstrates the cryptic role that developmental processes can have on ecology and trait evolution.

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