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The tradeoffs associated with leg regeneration in stick insects (Insecta: Phasmidae)

Tara Lynne Maginnis
The University of Montana

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THE TRADEOFFS ASSOCIATED WITH LEG REGENERATION IN STICK INSECTS (INSECTA: PHASMIDAE)

by

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B.S. University of Montana, 1998
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presented in partial fulfillment of the requirements for the degree of Doctorate of Philosophy

The University of Montana

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Tradeoffs are fundamental to evolutionary biology because they often link the expression of multiple traits, impeding the independent evolution of either trait. Conventional cost/benefit studies focus on life history traits, but many other systems or processes not usually thought of in the context of tradeoffs can generate a relevant dependence among traits. One example is appendage regeneration. The re-growth of a lost appendage requires the allocation of resources that would have otherwise gone to growth or reproduction, and can thus have associated costs.

Phasmids are unusual among insects in that they regularly shed and regenerate legs lost to fouled molts or predation attempts. Here I show how this process has profound consequences for these animals, and that the nature of leg regeneration tradeoffs differs among species. In a wingless species, leg regeneration reduced fecundity. In a winged species, leg regeneration has no effect on fecundity. Instead, leg regeneration stunted wing growth and hindered flight performance. Furthermore, rates of leg regeneration were common in natural settings (~25% of animals), suggesting stick insects regularly experience these biologically relevant tradeoffs. Explicit consideration of the integrated nature of animal phenotypes, in this case the non-independence of developing traits such as legs and fecundity or legs and wings, can be used to explain macro-evolutionary patterns in stick insects. Specifically, the interplay between the ecological conditions affecting the prevalence of leg loss and the allocation costs of leg regeneration may help to explain the repeated losses and gains of wings during stick insect evolution.

New approaches to studies of animal regeneration will provide further insight as to if and how the tradeoffs associated with regeneration have affected morphological evolution, or the evolution of regenerative capacities in general. In particular, future research that examines the tradeoffs associated with appendage regeneration in an evolutionary context promises to bring studies of this phenomenon full circle.
# Table of Contents

Acknowledgements ........................................................................................................ iv

List of Tables .................................................................................................................... v

List of Illustrations ......................................................................................................... vi

Chapter 1: Introduction .................................................................................................... 1

Chapter 2: Leg regeneration stunts wing size and hinders flight performance in a stick insect (Insecta: Phasmidae) .......................................................... 6

Chapter 3: Tradeoffs associated with leg regeneration in stick insects (Insecta: Phasmidae): two species, two different tradeoffs ............ 19

Chapter 4: The costs of regeneration: a review and framework for future research .......................................................... 48

Chapter 5: Field research with *Didymuria violescens*, the spur legged phasmid .......................................................................................... 81

Appendix 1: Supplemental flight results with *Sipyloidea sipylus* ................................ 102

Bibliography ..................................................................................................................... 111

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List of Tables

Effects of leg regeneration on fecundity in *C. morosus* and *S. sipylus* .......... 33
Species capable of appendage regeneration .................................................. 76
Body lengths of instars of *D. violescens* ........................................... 85
Frequencies of leg loss and regeneration in a population of *D. violescence* .... 89
Proportions of missing or regenerated legs that were front legs, mid legs, or hind legs (males and females combined) ............................................. 89
Summary of results from predation experiments on *D. violescence* .......... 91
Guidelines for rating individual descent performance .................................. 104
Summary of results on directional descent .............................................. 106
List of Illustrations

Sipyloidea sipylus, the pink winged stick insect........................................15
Leg regeneration stunted wing size and increased wing loading..................16
Leg regeneration impaired flight performance.............................................18
Leg regeneration in stick insects..........................................................43
Regenerated legs are smaller than non-regenerated legs............................44
Effects of regeneration on fecundity in C. morosus and S. sipylus...............46
Regeneration stunted wing size in S. sipylus...........................................47
Regeneration research compared with alternate mating tactics....................79
Male (left) and female (right) adult D. violescens.....................................95
Male and female D. violescens in copulation.............................................96
Missing legs and regenerated legs are easily identifiable in the field............97
An example of predator-free trees in Bago State Forest..............................98
Frequencies of leg loss and regeneration in adult D. violescens..................99
D. violescens leg lost to a spider web.....................................................100
Male D. violescens missing part of his abdomen, presumably due to a predator.101
Distance traveled during S. sipylus flight.............................................109
S. sipylus flight control............................................................................110

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"If there were no regeneration there could be no life.

If everything regenerated there would be no death."

Richard Goss, 1969
Chapter 1:

Introduction

Tradeoffs are fundamental to life history and evolution. For over a hundred years biologists have observed that organisms cannot be optimally adapted to all agents of selection at once, and must compromise aspects of some traits for others. A classic example is the tradeoff between number and size of offspring; often as the number of individual seeds/eggs an organism produces increases, the size of those seeds/eggs decreases. Indeed, tradeoffs are manifested in many aspects of behavior and physiology, and have influenced the evolution of plant and animal form and function.

One important type of tradeoff is the allocation tradeoff, where the allocation of resources to one trait comes at the expense of other traits. This interdependence among traits is often due to developmental resource competition, where several traits compete for limited resources. An intuitive way to study resource allocation tradeoffs is to quantify the costs associated with the production of a structure, and this has been profitably explored for the conspicuous morphologies of sexual selection; the allocation of resources to produce exaggerated ornaments and weapons has been shown to reduce life span, prolong development, and stunt the size of other morphologies. These studies clearly illustrate that investment into the production of major morphological structures is costly.

For my dissertation research I use a similar logic to explore the nature and magnitude of resource allocation tradeoffs. However, I capitalize on the remarkable tendency for
some animals to produce a morphological structure multiple times. Appendage regeneration provides a novel twist to allocation tradeoff research because animals faced with a lost appendage must grow a structure not once, but twice. Re-growing a lost appendage requires resources that, in principle, would have otherwise gone to growth or reproduction; thus, this process is associated with developmental resource competition tradeoffs. As such, taxa that routinely regenerate lost appendages are ideal for studying developmental resource allocation tradeoffs, and their potential importance to animal evolution.

Stick insects (Insecta: Phasmidae) are masters of regeneration. Commonly recognized for their long and thin shapes, these insects are nocturnal herbivores and include some of the most cryptic organisms in the world; they mimic their host plant in shape, color, texture, and sometimes even behavior, presumably due to a history of selection to reduce predation. Unusual among insects (but not arthropods in general; see Chapter 4), all phasmids regularly shed and regenerate legs. Legs can be lost to encounters with predators (e.g., if a predator grasps a leg instead of the body the animal can shed the limb and drop to the ground to escape predation), or can be lost to complications with molting. Stick insects molt upside down and use an elaborate series of twisting and turning maneuvers to free themselves of their old exoskeleton. During these molting events, the long and thin legs so effective at conferring crypsis may become trapped, and these tangled legs must be shed to survive. Complete replacement of a lost leg requires three consecutive molts, each molt producing a successively larger leg. Like most species with
regenerative capacities, the regenerated legs in stick insects never quite attain their full proportions (they end up ~10% smaller than non-regenerated legs).

In this study I characterize and quantify the tradeoffs associated with leg regeneration in several species of stick insect. Phasmids are a model system for exploring allocation tradeoffs because they are easily reared in the laboratory, and they vary in a major aspect of their morphology; approximately half of all species are fully winged and flight-capable, and half are partially winged or wingless. I show that these two different morphologies exhibit qualitatively different tradeoffs, and I suggest that these differences in the nature of regeneration-induced tradeoffs may have influenced stick insect evolution.

*Sipyloidea sipylus*, the pink winged stick insect, is a fully winged phasmid. Experiments described in Chapter 2 show that leg regeneration in this species stunts wing growth, and that this allocation tradeoff results in increased wing loading. These morphological consequences of leg regeneration translate into a significant reduction in several measures of flight performance, which may increase the risk of predation and/or injury in natural settings (see Chapter 2 and Appendix 1).

*Carasious morosus*, the common stick insect, lacks wings entirely. Experiments described in Chapter 3 show that leg regeneration in this wingless species leads to significant reductions in egg production – a profoundly different tradeoff resulting from the same basic developmental process. These results are noteworthy both because of the
magnitude of the effects, and because the nature of the allocation tradeoff differs between the two species.

Recognizing that leg regeneration has ecologically relevant costs, and that these costs vary depending on stick insect morphology, may help explain evolutionary patterns. A recent phylogenetic study of stick insects suggested they have lost and regained the ability to fly multiple times during their evolution. While other hypotheses exist to explain this pattern, my research provides a previously unconsidered explanation: tradeoffs associated with leg regeneration. In Chapter three, I describe how tradeoffs associated between leg regeneration and wings/fecundity may have influenced evolutionary patterns in stick insects.

While researching the costs associated with leg regeneration in stick insects, I performed an extensive survey of the literature to explore if and how my results compare to other studies in other taxa. This survey revealed that tradeoffs associated with appendage regeneration were much more common than previously considered, and that in most cases leg or tail regeneration was accompanied by performance or allocation costs. In Chapter Four, I review what is currently known about the tradeoffs associated with regeneration in both vertebrates and invertebrates, and use this review to suggest a shift in the way we ask and answer questions about the evolution of regenerative capacities.

Discussions about the tradeoffs associated with regeneration, and the evolution of regenerative capacities, both beg for information about how often regeneration occurs in
nature. In Chapter Five, I report results from a field study of a population of stick insects located in New South Wales, Australia. Results from this study demonstrated that leg loss and regeneration is surprisingly common in natural settings (~25% of sampled animals), and allowed me to successfully design biologically relevant experiments that could be conducted in the laboratory (see Chapter 2). Moreover, it led to the discovery that leg loss and regeneration decrease survivorship in the wild, something that would have been impossible to study in the laboratory. To my knowledge, it is the first comprehensive field survey of regeneration in any free-living phasmid population.

Appendage regeneration provides a new twist to allocation tradeoff research. Further exploration of the tradeoffs associated with appendage regeneration, particularly in an appropriate evolutionary context, promises to bring studies of this phenomenon full circle. Although the specific mechanisms underlying regeneration tradeoffs in stick insects may differ from the mechanisms in other systems, both the ecological implications and the concept itself are generalizable. In particular, the idea that traits besides the regenerated appendage can be affected by the regeneration process promises new insights for all taxa that regenerate, and again establishes the critical role that tradeoffs play in the evolution of animal form. By rigorously demonstrating that regeneration has measurable costs, and that these costs differ in predictable ways, this study may serve as a model system for examining tradeoffs associated with appendage regeneration, and may help explain the evolution of regenerative capacities in general.
Chapter 2:

Leg regeneration stunted wing growth and hindered flight performance in a stick insect (Insecta: Phasmida)

Major morphological structures are sometimes produced not once, but twice. For example, stick insects routinely shed legs to escape a predator or tangled molt, and these legs are subsequently re-grown. Here I show how appendage regeneration can be used as a novel tool for studying tradeoffs in biology; in Sipyloidea sipylus, the re-growth of a leg resulted in a reduction in wing area and an increase in wing loading. These morphological consequences of leg regeneration led to significant reductions in several biologically relevant measures of flight performance. This previously unrecognized tradeoff between leg regeneration on wing size and flight capability reveals how morphology, behavior, and habitat can interact to shape the evolution of animal morphology.

Tradeoffs are fundamental to evolutionary biology because they often link the expression of multiple traits, impeding the independent evolution of either trait (Stearns, 1992; Roff, 1992). One effective way to study tradeoffs is to quantify the consequences of allocating resources to the production of a trait. Although it is obvious that all developing structures require energy and resources as they grow, it is generally not clear how costly this investment is to the animal itself. Many of the best measures of the costs of trait production involve unusually large or conspicuous traits, presumably because they are
accompanied with correspondingly large, and therefore measurable, costs. Indeed, the allocation of resources to develop exaggerated ornaments and weapons of sexual selection in many birds, mammals, and insects can be accompanied with physiological costs (Møller, 1989; Nihjout and Emlen, 1998; Johnson, 1999; Emlen, 2001). I demonstrate an alternative approach to 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 and Bryant, 1985), fish (Wagner, 1982), and arthropods (Needham, 1953; Needham, 1965; Bulliere and 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. Because appendage regeneration requires the allocation of resources, this process can have profound consequences. In some lizards for example, re-growing a lost tail significantly reduced fecundity (Smyth, 1974; Maiorana, 1977; Dial and Fitzpatrick, 1981; Norman and Jones, 1993). However, tradeoffs between somatic and reproductive growth are not the only types of tradeoffs that can affect fitness. Here I show for a species of stick insect, Sipyloidea sipylus (Westwood, Figure 1), that the cost of producing a leg a second time can affect animal shape, and hence animal performance.

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 (Carlberg, 1986; Brock, 1999). Legs can also be
lost to complications with molting. Stick insects molt upside down and separate themselves from their old exoskeleton with elaborate twisting and turning maneuvers. During molting, legs can become entangled in the old cuticle and must be shed to survive (Brock, 1999; Maginnis, personal observation). After a leg is lost to a fouled molt or predation event, it is regenerated (immature stick insects always commence regeneration). Complete regeneration of a leg requires three consecutive molts, each producing a successively larger version of the replacement leg (Ramme, 1931; Wilbert, 1953; Carlberg, 1992).

A replacement leg in S. sipylus, the pink winged insect, comprises approximately 10% of the total weight of an animal (\( \bar{X} \pm \text{S.D.} = 0.132 \pm 0.027 \) gms). In a laboratory rearing experiment with random administration of treatments, leg regeneration stunted the growth of wings; individuals regenerating a right hind leg during development experienced an \(-15\%\) decrease in wing area compared to control animals that did not regenerate any legs (1-Factor ANCOVA, \( F = 29.916; p < 0.0001, \) Figure 2). This regeneration-induced decrease in wing size increased wing loading (1-Factor ANOVA, \( F = 73.306; p < 0.0001, \) Figure 3), and negatively impacted several ecologically relevant measures of flight performance.

Stick insects use their wings primarily to control the speed and trajectory of a free-fall descent (Carlberg, 1984; Dudley, personal communication; Maginnis, personal observation). 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
decent and land on the ground without injury, and a combination of gliding and active
flapping permits an individual to land on vegetation well above the ground. 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 order to test the hypothesis that stunted wings negatively affects aspects of flight
performance, free-fall descents were simulated in the laboratory by releasing individuals
from an elevated position (15 feet). Two aspects of every descent were recorded: 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 or flapping, and
impact of landing represented an additional index of decent control. Individuals in­
control of their descent presumably land dorsal side up: individuals out-of-control land
ventral side up or on their side (e.g., a ‘crash’ landing). In repeated trials, individuals that
regenerated a leg consistently fell faster than individuals that had not regenerated a leg (t
= 6.909; p < 0.0001; Figure 3). In addition, a higher proportion of regenerated
individuals experienced a ‘crash’ landing (45.2% compared to only 11.8% of control
animals), and ‘crash’ landings were correlated with time aloft (r = -0.644; p < 0.0001).
These results demonstrate that tradeoffs between leg regeneration and wings are
associated with biologically relevant reductions in flight performance (additive measures
of flight performance are described in Appendix 1).

In natural settings, the consequences of reduced flight performance may be even more
pronounced. Stick insects are arboreal herbivores and live high in the forest canopy.
Whether they are foraging, searching for mates, or escaping from predators, they may regularly descend from heights much greater than 15 feet; the consequences of leg regeneration on flight performance in these situations may be much more severe. Moreover, in vegetatively dense habitats, a relatively quick descent can lead to injury if animals strike branches during their fall. Coupled with the risk of injury upon landing, quick and uncontrolled glides or flights could likely increase the risk of predation or injury. Both of these observations suggest that leg regeneration, when it occurs, can have important performance consequences in natural populations.

Rates of regeneration are nontrivial 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]). High rates of regeneration in the wild suggest that the morphological tradeoffs resulting from leg re-growth may be pervasive and important agents of selection on stick insect populations. Consideration of these tradeoffs, as well as how often they are likely to occur, may help explain the macro-evolutionary patterns of morphological evolution in stick insects.

A recent phylogenetic study of stick insects showed multiple losses and regains of wings; after an initial loss of wings early in stick insect evolution, four lineages re-gained fully functional wings, and one of these lineages experienced two subsequent evolutionary losses of wings (Whiting et al., 2003). The authors suggested two factors that could have influenced wing evolution: tradeoffs between wings and fecundity and tradeoffs...
between wings and effective crypsis. In many hemimetabolous insects, wingless females have higher fecundity than winged females (Zera, 2000), and stick insects without wings may be more cryptic (Whiting et al., 2003). My study suggests an important and previously unconsidered explanation for this pattern: tradeoffs between wing development and leg regeneration.

Insect wings and wing musculature are notoriously expensive structures to build and maintain (Zera, 2001), and if the performance benefits (e.g., dispersal, foraging, mate location, etc.) of wings are not large, then these costs can favor the evolutionary loss of wing expression. The benefits of stick insect wings are not large to begin with; phasmid wings are relatively small for their body sizes, and these insects are primitive 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 possibility that wing production in regenerating animals will no longer be cost effective. If true, then habitats leading to high rates of leg loss may select for suppression of wing development. Two ecological circumstances are likely to lead to excessive natural rates of leg loss. Environments with high densities of predators, or in which stick insects fail to effectively hide (e.g., poor crypsis), could result in predator-induced leg loss in a large number of developing animals. Similarly, high predation rates favoring extreme crypsis may lead to the evolution of body morphologies so slender that legs will often become tangled during molting events. In both situations, animals would experience high rates of leg loss; because leg regeneration stunts wing growth and impairs flight performance, these habitats may favor the evolutionary loss of wings.
However, habitat also dictates how necessary wings are to foraging, mate location, and predation escape. In environments where canopy heights are especially high and resources are widely distributed, it is likely that even relatively poor flight will substantially improve fitness, rendering the development of wings more beneficial than their costs; these scenarios, especially if coupled with low rates of leg loss/regeneration due to molting or predation, could likely lead to an evolutionary re-gain of wings.

Tradeoffs between two very different process, leg regeneration and wing development, influence the final shape and flight performance of stick insects. This interplay between morphology, predation, and habitat structure can determine the ecological ‘price’ that animals pay to regenerate, and may have influenced the losses and/or regains of wings that occurred repeatedly during stick insect evolution. More research into the mechanisms behind this phenomenon will allow for further exploration into how the compromises associated with regeneration have interacted with the ecology of stick insects to influence their morphological evolution. This study documents a novel consequence of regeneration in insects, and demonstrates the cryptic role that developmental process can have on ecology and trait evolution.
Methods

Tradeoffs with wings:
Eggs of *Sipyloidea sipylus* 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 (0 or 1 leg regenerated). ’Regenerated’ animals were induced to regenerate their right hind leg at the beginning of the second instar; 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*). Fourteen animals lost additional legs during the experiment (from molting complications) and were not considered in subsequent analyses (14/50 = 28%). Upon completion of the flight experiments (see below), 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. Wing loading was calculated as [wing area / body size]. Relative wing sizes and wing loadings were compared for the two treatment groups using Analysis of Covariance (wing area or wing load as the dependent variable; body length as the covariate).

Tradeoffs with flight performance:
Within five days of eclosion, individuals were released from a 12-foot ladder (height of release was 15 feet off of the ground). Each individual was released five times, and a second observer recorded time aloft and impact of landing (safe, controlled landing vs. ’crash’ landing). A ‘crash’ landing was defined as a landing in a position other than dorsal side up. Average time aloft and the proportion of crash landings were compared
for treatment groups using unpaired t-tests assuming unequal variance. The relationship between average time aloft and proportion of crash landings was examined using a correlation test ($r$).

**Regeneration rates in the wild:**

To determine the rate of leg regeneration in a natural population, 803 adult *Didymuria violescens* (411 females, 392 males) were collected in Bago State Forest, New South Wales, Australia. Body size and length of all six legs were measured using digital calipers. Regenerated legs are always smaller than non-regenerated legs even after they have completed development (Carlberg, 1992; Ramme, 1931, Wilbert, 1953), so regenerated legs were readily identified by eye.
Figure 1: *Sipyloidea sipylus*, the pink winged stick insect.
Figure 2: Leg regeneration stunted wing size and increased wing loading. Nymphs that regenerated a right hind leg developed into adults with proportionately smaller wings than nymphs not regenerating a leg (1-Factor ANCOVA, \( F = 29.916, p < 0.0001, 'a' \)). This translated into a significant increase in the wing loading of these individuals (1-Factor ANOVA, \( F = 73.306, p < 0.0001, 'b' \)).
(b)

Wing Load
(wing area / body size)

<table>
<thead>
<tr>
<th></th>
<th>0 legs regenerated</th>
<th>1 leg regenerated</th>
</tr>
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<tbody>
<tr>
<td>Wing Load (kg)</td>
<td>1.0</td>
<td>1.2</td>
</tr>
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* indicates a significant difference.
Figure 3: Leg regeneration impaired flight performance. Individuals that regenerated a right hind leg during ontogeny, and hence developed proportionately smaller wings, fell faster during free-fall descents than control animals ($t = 6.909; p < 0.0001$). Individuals regenerating a leg during development descended at an average rate of 1.6 seconds (S.E. $\pm 0.07$); control individual descended at an average rate of 2.7 seconds (S.E. $\pm 0.13$).
Chapter 3:

Tradeoffs associated with leg regeneration in stick insects (Insecta: Phasmida): two species, two different tradeoffs

Abstract

Stick insects regularly shed and regenerate legs lost to fouled molts or predation attempts. Here I demonstrate that this phenomenon is associated with developmental resource-allocation tradeoffs, and that the nature of these tradeoffs differs among stick insect species. In Carasious morosus, a wingless species, regeneration of a hind leg significantly reduced fecundity. In Sipyloidea sipylus, a flight-capable winged species, regeneration did not affect fecundity. Instead, it led to a reduction in relative wing size. Explicit consideration of the existence and nature of appendage growth tradeoffs may help explain evolutionary patterns in stick insects, including repeated evolutionary losses and gains of wings.

Introduction

Tradeoffs are an almost universal feature of life. One important type of tradeoff arises when the allocation of critical or limiting resources to one purpose during development comes at the expense of an individual’s ability to allocate resources to other purposes. In 1818, Goeffroy St. Hilaire first described this phenomenon as a “loi de bâncement”. This process was later described by others as the “struggle of parts” (Roux, 1881), the “law of compensation” (Darwin, 1899), and “material compensation” (Rensch, 1959).
all cases, this form of tradeoff revolved around the fundamental inability of developing organisms to simultaneously invest at optimal levels in the production of all traits. Limitations in the availability of critical developmental resources caused enhancements to the size of one structure to come at the expense of growth of other structures.

Sexually selected traits provided some of the clearest examples of the tradeoffs that can accompany the production of morphological structures, in part because individual investment into the production of these traits can be so extreme. Many birds, mammals, and insects produce exaggerated traits that are used in male-male competitions or as ornaments to attract females. The elaborate tails of peacocks, immense antlers of ungulates, and defense appendages in arthropods can drastically improve the mating success compared to individuals who lack these exaggerated structures (Darwin, 1871; Andersson, 1994). Although beneficial for reproduction, these enlarged traits can also cost animals in a variety of ways, and it is ultimately the balance between these benefits and costs that shape patterns of their evolution. Exaggerated traits can decrease foraging efficiency (Møller, 1989), impair locomotion (Balmford et al, 1993; Johnson, 1999; Møller, 1999) and/or increase the risk of predation (Lloyd, 1984; Møller and Nielsen, 1997). Moreover, the energy expended to develop an exaggerated morphology may require the allocation of resources that would have otherwise gone to growth or reproduction.

Expensive traits need not be flashy or exaggerated; features besides sexually selected traits can also be costly. For example, the development of a ‘regular’ trait (e.g., wings)
can result in allocation tradeoffs with ovaries and testes, and hence reproduction (Zera, 2000; Stirling et al., 2001). Indeed, resource allocation tradeoffs between somatic growth and reproduction provide one of the foundations of life history theory (Stearns, 1992; Roff, 1992), and these tradeoffs are thought to have shaped much of life history evolution.

Although growth versus reproduction tradeoffs are intuitive, and are often presumed to be universal, they can be very difficult to quantify. Some of the best empirical measures of the costs of trait production come from species that only sometimes produce the trait. Facultative trait production (polyphenism), means that genetically similar individuals (e.g., siblings) can be induced to develop along very different alternative trajectories (e.g., with and without wings). Comparing the relative fecundities of these otherwise similar individuals can reveal allocation costs associated with trait production.

Crickets, for example, can develop into one of two adult forms depending on their social environment. Nymphs developing in relatively crowded conditions develop functional wings and wing muscles as adults, which can comprise up to 30% of an individual's biomass, and these animals have large quantities of lipids to support flight; larvae developing in un-crowded conditions lack functional wings and wing muscles, and have reduced lipid stores (Zera, 2000). Thus, environmental conditions encountered during nymphal development (e.g., crowding) trigger one of two alternative patterns of gene expression, and result in adults with dramatically different morphologies, physiologies, and behavior. Specifically, these alternative phenotypes store, mobilize, and utilize
energy resources very differently. In long-winged crickets, nutrients are biosynthesized into lipids for use as flight fuel; in short-winged crickets, nutrients are biosynthesized into yolk protein for eggs (Zera, 2000). These differences in the allocation of acquired nutrients result in a substantial tradeoff between wings and fecundity: long-winged females have reduced fecundity compared to short-winged females. This pattern, named the "flight-fecundity syndrome", has been observed in many insects, and is presumed to have shaped multiple aspects of insect life history, morphology and behavior (Roff, 1992).

Behavior tradeoffs associated with exaggerated morphologies (e.g., ornaments and weapons) or tradeoffs between somatic growth and reproduction (e.g., cricket wings), are not the only types of tradeoff that can influence the evolution of animal form and function. A less well-studied type of tradeoff arises whenever allocation to the production of one structure negatively impacts the relative sizes of other structures (Nijhout and Wheeler 1996; Klingenberg and Nijhout 1998; Nijhout and Emlen 1998; Emlen 2000, 2001; Moczek and Nijhout 2004). That is, tradeoffs can remain solely within the arena of somatic growth, and directly influence animal shape. This interdependence among morphological traits is especially well studied in insects. For example, butterflies develop wings that are proportionately much larger than the wings of most other insects, and forewings and hindwings compete for the same pool of available resources: surgical removal of early-stage developing hindwings can increase the relative size of forewings (Klingenberg and Nijhout, 1998; Nijhout and Emlen, 1998), a trait that is important to both natural and sexual selection (Frankino et al., 2005). Similarly, many
stag beetles with enlarged mandibles develop proportionately smaller wings; as mandible size increases, relative wing size decreases (Kawano, 1997). These traits appear to be directly competing for common and limiting resources during development, and this results in an allocation tradeoff between the two morphological structures. Both of these examples show a clear negative correlation between the relative sizes of traits, and illustrate how competition for resources may affect animal proportions, and therefore body shape. In all these examples (e.g., sexually selected traits, cricket wings, beetle mandibles, etc.), the clearest measures of fitness costs are provided by comparisons between individuals that do, and do not, produce a structure.

In this study I capitalize on a different yet naturally occurring phenomenon that can also cause genetically similar individuals to vary in their allocation to the production of a morphological trait. Many animals readily shed an appendage during development (autotomy) to escape predation or some otherwise potentially fatal incident (e.g., the reflexive loss of a lizard tail in the face of a predator [Dial and Fitzpatrick, 1983; Daniels et al, 1986; Formanowicz, 1990] or the loss of entangled legs during arthropod molting events [Robinson et al, 1991; Brock, 1999; Maginnis, personal observation]). After autotomy, many of these animals subsequently grow the appendage a second time. Regeneration provides a novel twist to tradeoff research because growing a structure twice can be just as costly as the original growth of disproportionately large or exaggerated traits such as wings, ornaments, or weapons. As such, species that routinely regenerate lost appendages can be used as a unique tool to study developmental resource allocation tradeoffs, and their potential importance to animal evolution.
As with exaggerated morphologies, regenerated appendages are accompanied by basic
behavioral costs. In almost all taxa known to regenerate, regenerated appendages do not
attain their full proportions. Regenerated appendages of lizards and amphibians (Hardy
and Hardy, 1977; Daniels, 1983; Salvador et al, 1996; Fitch, 2003), crustaceans
(Edwards, 1972; Savage, 1975; Elner, 1980; Weis, 1982), fish (Becerra et al, 1996; Mari-
Beffa, 1999), insects (Lüscher, 1948; Wolsky, 1957; Parvin and Cook, 1968; Tanaka and
Ross, 1989; Karuppanan, 1998), and spiders (Vollrath, 1990) are smaller than the
originals. These reduced appendages can impair foraging (Elner, 1980; Vollrath, 1987;
Leonovich and Belozerov, 1992; Krink and Vollrath, 1999; Schneider and Vollrath,
1998), survivorship (Savage, 1975; McVean and Findlay, 1979; Wilson, 1992; Liu and
Wang, 1999; Lysenko et al, 2000; Fox and McCoy, 2000), and/or behaviors related to
mating/sexual selection (Sekkelsten, 1988; Lakes and Müche, 1989; Salvador et al, 1995;
Salvador et al, 1996; Uetz et al., 1996). Similar to the physiological development of
wings and wing muscles in the cricket system, the re-growth of a lost appendage can be
accompanied with fecundity tradeoffs. In some lizards and worms for example,
regeneration significantly reduces fecundity (Smyth, 1974; Maiorana, 1977; Dial and

Here I show for two species of stick insect, Carasious morosus and Sipyloidea sipylus
(Insecta: Phasmidae), that the allocation of resources to re-grow a lost appendage can
negatively affect the growth of other traits; the re-growth of a lost appendage, an aspect
of somatic growth, led to tradeoffs with reproductive growth in a wingless species, and to
tradeoffs with other morphological structures in a winged species. These results are noteworthy both because of the magnitude of the effects, and because the nature of the allocation tradeoffs differed between the two species. I discuss these results in the context of recent studies of stick insect evolution, and suggest how developmental resource allocation tradeoffs may have influenced wing evolution in these insects.

Background: Stick insects and leg regeneration

Stick insects are arboreal herbivores and include some of the most cryptic organisms in the world. There are approximately 4,000 described species, most of which live in tropical regions (Brock, 1999). They range from just over 2 cm in length to over 20 cm, and many possess large spines or leaf-like appendages, all thought to be the result of a history of natural selection to match their host plant in color, texture, and shape (Bedford, 1978; Brock, 1999). In addition to their cryptic morphology, stick insects can mimic plant behavior; they can sway like a branch in the breeze or feign death like a fallen twig (Carlberg, 1986; Brock, 1999, Maginnis, personal observation).

All phasmids readily shed and regenerate limbs. In natural populations, legs can be lost to complications with molting or to encounters with predators. Stick insects molt hanging upside down, and must separate themselves from their old exoskeleton with elaborate twisting and turning maneuvers. During this process, the exceptionally long and thin legs can become entangled in the old exoskeleton, and these legs must be shed if the animal is to survive (Brock, 1999; Maginnis, personal observation). Legs can also be lost to encounters with predators; if a bird or mantid or other predator grabs a leg instead
of the body, the leg may be shed and the animal can drop to the ground to escape predation (Carlberg, 1986; Brock, 1999; Maginnis, personal observation). ‘Predation events’ need not be typical; legs may also be lost to the viscous surface of a spider web or fresh sap flow (Maginnis, personal observation). Regardless of which section of the leg is entangled or trapped, legs are always autotomised at the coxa-femur junction (Maginnis, personal observation). If a portion of a leg is forcefully removed (e.g., a bird severs the leg at mid-femur), animals will shed the remaining portion of the leg at the coxa-femur junction within a few days (Maginnis, personal observation).

Phasmids are hemimetabolous (incompletely metamorphic) developers, and require between 5 to 8 nymph-nymph molts and a nymph-adult molt to complete development (the number of molts to complete development is not facultative on the species level, but does vary among species, Maginnis, personal observation). Autotomized legs begin regeneration from clusters of epidermal cells in the base of the leg coxa (Ramme, 1931). Replacement legs require three consecutive molts to fully regenerate; the first molt after leg-loss produces a leg one-fourth the normal size, the second molt produces a leg one-half the normal size, and the third molt produces a leg almost normal in length and width (Ramme, 1931, Figure 1). Nymphs commence regeneration even when there are insufficient remaining molts to complete the process, and adults are incapable of regenerating lost legs.

Stick insects provide an ideal opportunity for measuring tradeoffs associated with appendage growth for three reasons. First, leg regeneration is a natural phenomenon that
readily occurs in the wild (e.g., ~25% of over 800 adults in an Australian population were observed to have regenerated a leg during development [see Chapter 5]). Second, autotomy is easy to induce in the laboratory, permitting comparisons between individuals that have, and have not, re-grown a leg. And third, although all known stick insect species shed and regenerate legs, not all species are expected to compensate for these losses in the same way. Specifically, morphological differences among stick insect species may, and does, influence the nature of resource allocation tradeoffs associated with leg development.

Although most stick insects have a characteristically slender – often extremely thin – shape, species differ in the production of wings. About half of described phasmid species lack wings completely, or have only vestigial/ornamental wings (flight-incapable), and the rest produce fully developed wings and are capable of flight (Whiting et al, 2000). These two stick insect morphologies provide an excellent template for exploring how one developmental process, in this case leg regeneration, can lead to different resource allocation tradeoffs based on animal shape. In this paper I characterize leg regeneration-induced tradeoffs in two stick-insect species, one wingless, the other fully winged, and show that leg regeneration has profoundly different morphological and life history consequences in the two species. In the wingless species leg regeneration leads to reductions in fecundity, while in the winged species it leads to reductions in relative wing size.
Methods

Rearing and Regeneration Treatments

Approximately 75 eggs of *Carasious morosus* (de Sinety) the common or Indian stick insect, and 75 eggs of *Sipyloidea sipylus* (Westwood), the pink winged stick insect, were obtained from commercial breeders and reared in growth chambers (Day = 12:12 L:D cycle, 26:24°C). Both of these species readily reproduce parthenogenetically in the laboratory (females lay viable eggs in the absence of males), and all eggs obtained for use in these experiments were eggs produced from parthenogenesis. The first 50 nymphs of each species to emerge from their eggs intact were used for the experiment (legs can be lost as the animal hatches from the egg). As nymphs emerged, they were randomly assigned to one of two treatment groups: 0 or 1 leg regenerated during ontogeny. ‘Regenerated’ animals were induced to regenerate their right hind leg at the beginning of the second instar; pinching a leg with forceps causes the animal to drop the leg and commence the regeneration process. Individuals were reared in ten large mesh cages (2’ x 2’ x 4’), with approximately ten individuals in each cage. The large cages ensured that animals had sufficient space to molt, and minimized the chances of legs being lost to molting complications. All individuals were inspected daily for additional leg losses, and any individuals that spontaneously lost legs during molting were removed from the experiment. Animals were misted twice daily with water and fed *ad libitum* (bramble, *Rubus idaeus*) until maturity.
Measuring Fecundity and Morphology

Upon maturity, females were removed from the large mesh cages and placed into individual cages (half gallon plastic containers with ~ 25 holes for circulation, covered with mosquito netting to ensure the containment of all eggs). Individuals were fed *ad libitum* until they began to lay eggs. Again, females of both species are parthenogenetic, so no males were used for mating. For the first 30 days after the commencement of egg-laying individuals were fed *ad libitum*. On the thirtieth day of egg laying, all food was removed and females were kept under food-limited conditions for a period of 10 days. Eggs were collected and counted daily for the entire assessment period (40 days). Fecundity measures were compared for the two treatment groups (0 or 1 leg regenerated during ontogeny) using Analyses of Variance. Individuals that died during the fecundity experiments were not included in the fecundity analyses, but these animals were included in morphometric analyses.

After completion of the fecundity measurements, animals were euthanized. All six legs were removed from each adult female and, when appropriate, the wings were also removed. Legs were severed at the coxa-femur junction (the site of autotomy in living animals), and wings were removed at their base. Hind wings were unfolded and mounted on poster board for measurement (in *S. sipylus* the hind wings are fully functional and the front wings are rudimentary tegmina that are less than 1/20\(^{th}\) the size of the hind wing). Total body length (head plus thorax plus abdomen), length of each leg, and hind wing area were measured using a Leica stereomicroscope equipped with an ocular micrometer and Scion Image software. Wing areas were compared between the two treatment
groups using Analyses of Covariance, with body size as the covariate. Two comparisons were made to determine the effects of right hind leg regeneration on right hind leg size: comparisons between right and left hind legs within regenerating animals (paired t-test assuming unequal variance), and comparisons between right hind legs in control animals and right hind legs in regenerating animals (Analysis of Covariance, with body size as covariate).

Results

Breeding results

*Carasius morosus*

Nine individuals lost a leg while hatching from the egg (n$_{Total}$ = 59). After hatching, individuals required approximately 90 days to become adults (X ± S.D. = 87.4 ± 10.3 days). Animals underwent six molting events during this process: 1$^{st}$ instar 7.5 ± 1.1 days, 2$^{nd}$ instar 12.4 ± 1.9 days, 3$^{rd}$ instar 12.6 ± 2.1 days, 4$^{th}$ instar 13.3 ± 2.0 days, 5$^{th}$ instar 19.4 ± 3.0 days, 6$^{th}$ instar 22.1 ± 1.8 days, adult > 50 days.

Over the course of the experiment, 12 individuals lost additional legs after treatments had been administered, and were subsequently excluded from the final analyses. In all cases, these resulted from complications that arose during molting: 4 animals from the control treatment group and 8 animals from the 'regenerated' treatment group. Of these 12 individuals, 5 lost front legs, 4 lost middle legs, and 3 lost hind legs. Adult females ranged from 70.62 – 83.46 mm in length (X ± S.D. = 77.06 ± 3.82 mm), and these animals began to lay eggs 10 - 15 days after their final molt (X ± S.D. = 12.1 ± 1.8
days). Survival rate of individuals used throughout the entire experiment (e.g., from hatching to the end of the fecundity experiment) was 76.3% (n = 38), and survival rates did not differ between treatment groups.

*Sipyloidea sipylus*

Four individuals lost a leg during hatching (n_{Total} = 54). Upon hatching, individuals required approximately 100 days to become adults (\( \bar{X} \pm S.D. = 103.2 \pm 12.6 \) days). Animals underwent seven molting events to complete this process: 1\(^{st}\) instar 2.9 ± .8 days, 2\(^{nd}\) instar 9.1 ± 3.0 days, 3\(^{rd}\) instar 12.0 ± 1.8 days, 4\(^{th}\) instar 13.9 ± 3.1 days, 5\(^{th}\) instar 19.9 ± 3.2 days, 6\(^{th}\) instar 22.4 ± 2.0 days, 7\(^{th}\) instar 23.0 ± 2.0 days, adult > 50 days.

Over the course of the experiment, 14 individuals lost additional legs due to molting complications: 8 animals from control treatment group and 6 animals from the ‘regenerated’ treatment group. Of these fourteen individuals, 5 lost front legs, 3 lost middle legs, and 6 lost hind legs. Adults were between 87.41 – 96.74 mm in length (\( \bar{X} \pm S.D. = 92.13 \pm 2.63 \) mm). Adults begin to lay eggs 10-15 days after their final molt (\( \bar{X} \pm S.D. = 13.1 \pm 2.6 \) days). Survival rate of individuals used throughout the entire experiment was 80.6% (n = 36).
Effects of hind leg regeneration on hind leg size

In both species, regenerated hind legs at the completion of development were smaller than non-regenerated hind legs. This was evident from comparisons within regenerating animals of the relative sizes of the regenerated leg (right-side hind leg) and the opposite leg (left-side hind leg, C. morosus: paired t = -16.9993, p < 0.0001; S. sipylus: paired t = -16.945, p < 0.0001). It was also evident from comparisons across experimental treatments between right-side hind legs in regenerating animals and right-side hind legs in control/non-regenerating animals (C. morosus: 1-Factor ANCOVA, F = 24.578, p < 0.0001, Figure 2; S. sipylus: 1-Factor ANCOVA; F = 44.816, p < 0.0001, Figure 2). Overall, regenerated hind legs of both species were approximately 10% smaller than non-regenerated hind legs.

Effects of hind leg regeneration on fecundity

Carasious morosus

Regeneration in the wingless species significantly reduced fecundity; during both ad libitum and food limited conditions, regenerating animals laid fewer eggs per day compared to control animals (ad libitum: 1-Factor ANOVA, F = 4.045, p = 0.0544; food limited: 1-Factor ANOVA, F = 4.605, p = 0.0410). During the first 30 days, C. morosus laid an average of one egg per day, and occasionally as many as six eggs per day; this average dropped to nearly zero during the food-limited period (Table 1). In fact, among the 14 individuals that regenerated during development, only one individual laid eggs during the food-limited period, and that female only laid one egg. Over the entire 40
days, leg regeneration reduced fecundity by approximately 25% (1-Factor ANOVA, F = 5.218, p = 0.0304, Figure 3).

*Sipyloidea sipylus*

Regeneration in the winged species had no net effect on fecundity. During the *ad libitum* food treatment, there was a trend for individuals who regenerated during development to lay fewer eggs (1-Factor ANOVA, F = 3.229, p = 0.0835). However, during the food limited period (the final 10 days), there was a trend for regenerated individuals to lay more eggs (1-Factor ANOVA, F = 3.729, p = 0.0604). Over the entire 40 days, regeneration had no overall effect on fecundity (1-Factor ANOVA, F = 1.90, p = 0.6667, Figure 3). Throughout the experiment, *S. sipylus* consistently laid an average of two eggs per day (Table 1).

Table 1: Effects of leg regeneration on fecundity in *C. morosus* and *S. sipylus*.

<table>
<thead>
<tr>
<th></th>
<th>Average # of eggs laid per day</th>
<th></th>
<th>Cumulative</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>30 days</td>
<td>10 days</td>
<td></td>
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<tr>
<td></td>
<td><em>ad libitum</em></td>
<td><em>food limited</em></td>
<td></td>
</tr>
<tr>
<td><em>C. morosus</em> - control</td>
<td>1.17 (±.69)</td>
<td>0.31 (± .22)</td>
<td>1.01 (±.77)</td>
</tr>
<tr>
<td><em>C. morosus</em> - regenerated</td>
<td>0.70 (± .57)</td>
<td>0.02 (± .006)</td>
<td></td>
</tr>
<tr>
<td><em>S. sipylus</em> - control</td>
<td>2.18 (± .30)</td>
<td>1.79 (± .31)</td>
<td>4.07 (±.52)</td>
</tr>
<tr>
<td><em>S. sipylus</em> - regenerated</td>
<td>1.98 (± .29)</td>
<td>2.07 (± .45)</td>
<td></td>
</tr>
</tbody>
</table>

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Effects of hind leg regeneration on wing size

_Sipyloidea sipylus_

Regeneration affected wing area in _S. sipylus_. Individuals induced to regenerate a right hind leg during development produced wings that were proportionately smaller than wings of individuals not regenerating a leg (1-Factor ANCOVA, _F_ = 29.916, _p_ < 0.0001, Figure 4). Across the range of body sizes, regenerated animals had approximately 15% smaller wings.

**Discussion**

Tradeoffs have been recognized since Darwin, when he suggested that ‘in order to spend on one side, nature is forced to economise on the other’ (Darwin, 1859, cited in Eberhard, 2003). In this experiment, I evaluated the effects of leg regeneration on both a wingless and winged species of stick insect to determine if and how regeneration forces the economization of somatic and/or reproductive growth. In _C. morosus_, a wingless species, regeneration decreased fecundity. In _S. sipylus_, a winged species, regeneration had no affect on fecundity but decreased wing area. These results suggest that there are developmental resource-competition tradeoffs operating between leg regeneration and fecundity, and leg regeneration and wing/flight muscle development. Because the nature of the tradeoffs associated with leg regeneration differed based on morphology, these tradeoffs may have contributed to phasmid evolution.
Many insects show fecundity costs due to the allocation of resources to other morphological structures. This is most often seen in the development of wings, and one of the correlates with wingless insects is increased fecundity (Zera, 2000, Roff, 1992). In these cases, the allocation of resources to develop and maintain wings and wing musculature appears to come at the direct expense of allocation to reproduction. In the wingless stick insect, the re-growth of leg can be compared to the development of wings and wing musculature; the allocation of resources to leg regeneration came at the expense of reproduction. Wingless individuals regenerating one leg during development experienced almost a 25% reduction in fecundity.

Previous studies of appendage regeneration in animals have documented tradeoffs specifically between regeneration and fecundity (Smyth, 1974; Maiorana, 1977; Dial and Fitzpatrick, 1981; Hill, 1989; Norman and Jones, 1993), suggesting that this may be a widespread cost of appendage re-growth. Interestingly, in phasmids, the tradeoff between regeneration and fecundity was only observed in the wingless species. Fecundity is usually a principle determinant of fitness in female animals, and is therefore an expensive price to pay for leg growth.

In the winged stick insect, the tradeoffs associated with leg regeneration were revealed in an entirely different arena. The winged species showed no tradeoffs between somatic and reproductive growth, but instead revealed tradeoffs between different morphological structures; the allocation of resources to re-grow a lost leg significantly reduced wing area. Although not as obvious as reductions in fecundity on its effects on fitness, reduced

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wing size is ecologically relevant. Stick insects primarily use their wings to slow and control free-fall descents. Whether they are foraging, searching for mates, or forced out of a tree by a predator or strong wind, a combination of gliding and active flapping allows an individual to maneuver through the canopy and land safely on a branch or the forest floor (Carlberg, 1984; Dudley, personal communication; Maginnis, personal observation). Based on a recent laboratory study of stick insect flight, the relatively smaller wings of animals that regenerate a leg significantly reduced several aspects of flight such as time aloft, landing ability, distance traveled, and overall flight control/performance (see Chapter 2 and Appendix 1).

Other studies have shown how developmental resource competition tradeoffs can affect animal shape. Many dung beetles, for example, develop enlarged horns. Beetle horns are used in male-male competitions, where the males guard and defend underground tunnels containing females. Horns can develop on the front of the head, the back of the head, or the front of the thorax, and the type of tradeoff associated with horn development differs based on the physical location of the horn. Beetles with horns on the front of the head develop relatively smaller antennae; beetles with horns on the back of the head develop relatively smaller eyes; beetles with horns on the front of the thorax develop relatively smaller wings (Emlen, 2001). Horn development appears to primarily impact the growth of physically adjacent or nearby structures, and thus impaired olfaction, vision, or flight. This example shows how the phenotypic expression of a morphological trait is not independent of other traits, and how tradeoffs can directly influence the range of phenotypes that can be produced.
This phasmid study is the first to report somatic/reproductive growth tradeoffs (e.g., leg regeneration and fecundity) and morphological tradeoffs (e.g., leg regeneration and wings) in a hemimetabolous insect. Not only does regeneration produce different tradeoffs based on stick insect morphology (e.g., winged versus wingless), but the nature of those tradeoffs varies with respect to the relationship between somatic and reproductive growth. This kind of variation in tradeoffs has however, been demonstrated in holometabolous insects. In the dung beetle system introduced above, horn development can also negatively affect gonad investment. Large males, and hence males with large horns, have relatively smaller testes than small males; the allometric relationship between testes and body size reveals small males investing proportionately more into testes size than relatively large males (Simmons and Emlen, in prep). Coupled with the effects that horn development can have on eyes, wings, or antennae, this system demonstrates how the tradeoffs associated with one developmental process can differ among species. That is, horn development is associated with both growth/reproduction tradeoffs and several different morphological tradeoffs.

Researchers studying the dung beetle system have used the tradeoffs associated with horn development to explain evolutionary patterns. Over time, beetle horns have diversified and radiated, often with many losses and regains of different horn locations (Emlen et al., in press). The authors suggested that the repeated losses and regains of horn types are best explained by selection to minimize the costs associated with horn development. Recall that horn development, in addition to its affects on reproduction, came at the expense of other structures and thus impaired olfaction, vision, or flight; the functional
cost of reduced smell, sight, or flight however, varies depending on habitat. For example, nocturnal beetle species depend heavily on their eyes to see in low light conditions; as predicted, significantly fewer nocturnal species have horns at the base of the head (and thus tradeoffs with eyes) compared to diurnal species (Emlen, 2001). This pattern persists through examinations of the need of wings for flight (e.g., dispersal distance based on population density), and antennae for smell (e.g., odor detection in open vs. dense habitats; Emlen et al., in press). The tradeoffs associated with horn development are directly related to their environment, and demonstrates how resource allocation tradeoffs have interacted with beetle ecology to directly influence their morphological evolution.

It is possible to use the tradeoffs associated with leg regeneration to explain evolutionary patterns in stick insects; because leg regeneration has measurable costs, and because the nature of these costs differs in species with different morphologies, tradeoffs associated with leg regeneration may explain the repeated loss and regain of wings that occurred during phasmid evolution. Whiting et al. (2002), suggested stick insects experienced an early evolutionary loss of wings, and then regained wings and wing musculature four separate and independent times during their evolution. In fact, in one of the evolutionary lineages that regained wings, there were an additional two evolutionary losses of wings (Whiting et al., 2002). The reversal from a flightless to volant form has never been documented in insects, and suggests the 're-evolution' of relatively complex structures may be more common than previously considered (Whiting et al., 2002). The authors suggested tradeoffs with crypsis and/or fecundity as potential evolutionary forces for the
independent losses and regains of wings; wingless species may be more cryptic than winged species, and energy used for the development of wings could be used for egg production (Whiting et al, 2002). Their second hypothesis is consistent with numerous studies that have documented reduced fecundity in the winged morph of winged-polymorphic insects (Zera, 2000, see above). In the two species studied here, not only does the winged phasmid lay relatively more eggs than the wingless (see Table 1), but regeneration affected fecundity in the wingless species and not the winged. These results suggest an additional benefit to wings; wings, in addition to their use for flight, could serve as a potential “sink” for any costs incurred during leg regeneration. That is, the consequences associated with regenerating a new, replacement leg will reside in the wings instead of eggs.

It also raises the possibility that the costs associated with leg regeneration have been an additional evolutionary force for the loss and regain of wings in some environments. One study has looked for rates of regeneration in natural habitats, and discovered ~25% of nearly 1000 adults had at least one regenerated leg (see Chapter 5); while this proportion might be typical of other species, rates of leg regeneration may be much different based on habitat and/or morphology. For example, habitats may differ in the intensity of the type of predation, affecting how often animals lose legs to predators. Similarly, selection for crypsis may favor different stick morphologies in different structural habitats, and habitats favoring especially slender body forms may be associated with higher frequencies of leg loss due to complications in molting. Habitats also likely dictate how necessary wings are to foraging, mate location, and/or predation escape; particularly high
and open canopies might render flight more useful than a lower, dense canopy. In conditions where canopy height and/or regeneration rates are high, it is possible the benefit of having wings (e.g., for flight and/or to absorb regeneration costs) outweighs the physiological cost of developing wings. Conversely, in conditions where regeneration rates and/or canopy heights are low, it is likely the benefit of not having wings (e.g., to increase crypsis), could outweigh the benefit of having wings for flight and/or absorbing the costs of regeneration. These and other factors are likely to influence the ecological 'price' animals pay to regenerate.

Some speculation has been made as to whether tradeoffs (either between growth and reproduction or between morphological structures) can be used to predict associations between morphology and ecology. That is, perhaps the ecological costs associated with a trait, rather than (or in addition to) the benefits, are driving the directions of morphological evolution (Emlen, 2001). In addition, questions remain regarding if and how these predictable patterns can be used to explain evolutionary changes in traits, such as the repeated losses and regains of beetle horn types or phasmid wings. In holometabolous insects, tradeoffs persist because two conditions are met. One, the 'expensive' developmental process (e.g., horn development) coincides with the timing of the development of adult morphology. And two, resources are limited for at least part of this period. In the dung beetle system, both criterion are met because horns are produced after the larvae ceases feeding, and stored resources are used to develop all adult morphologies (Emlen, 2001). In hemimetabolous insects, such as stick insects, the 'expensive' developmental process may or may not coincide with the development of the
adult morphology (chance determines leg loss and hence the timing of regeneration), and resources are used over time to develop adult morphologies (even with substantial time to recuperate or acquire nutrients, significant tradeoffs persist, e.g., leg regeneration and fecundity).

Results from this study demonstrate three important points related to the generalization of using tradeoffs to explain evolutionary patterns. First, a completely different developmental process can generate differences in functional costs (e.g., appendage regeneration instead of the development of an enlarged morphology). Second, tradeoffs can occur even when the expensive developmental process is separated in space and time from the adult morphology (e.g., hemimetabolous vs. holometabolous). This is consistent with flight-fecundity tradeoffs in crickets. The development of wings and eggs are uncoupled in time; the growth of flight muscles occurs before ovarian growth, thus the two do not appear to directly tradeoff with each other (Zera, 2000). And third, ecologically relevant tradeoffs can persist even when resources aren’t limited (e.g., ad libitum conditions still produce a tradeoff between leg regeneration and wing size and leg regeneration and fecundity). These three factors suggest that variation in the nature of tradeoffs based on expensive developmental processes is more widespread than previously considered, and that this variation may be used to explain evolutionary patterns.

Future research into the mechanisms behind leg regeneration in stick insects will allow us to explore if and how the tradeoffs associated with regeneration have shaped their
morphological evolution. In particular, the physiology of leg regeneration, egg
development, and flight muscle development is poorly understood in phasmids; research
into the genes and hormones used in these processes could shed light as to how the
tradeoffs between regeneration and wings/fecundity are physiologically manifested in
adults. In addition, including aspects of ecology into these developmental studies
promises to provide new insights into the ways that this physiological process might have
constrained or biased the directions of morphological evolution. This study documents
how one process, in this case regeneration, led to tradeoffs between somatic and
reproductive growth as well as tradeoffs between morphological structures. These
differences were dictated by morphology, and could help explain the evolutionary
patterns of wings in phasmids.
Figure 1: Leg regeneration in stick insects. The regeneration process in stick insects requires three consecutive molts. The first molt after leg loss produces a leg about $\frac{1}{4}$ the normal size; the second molt after leg loss produces a leg about $\frac{1}{2}$ the normal size; the third molt after leg loss produces a final leg approximately 10% smaller than it otherwise would have been.
Figure 2: Regenerated legs are smaller than non-regenerated legs. In both *C. morosus* (a) and *S. sipylus* (b), regenerated right hind legs (closed circles) are proportionately smaller than non-regenerated right hind legs (open circles; *C. morosus*: 1-Factor ANCOVA, $F = 24.578, p < 0.0001$; *S. sipylus*: 1-Factor ANCOVA, $F = 44.816, p < 0.0001$).

(a) *C. morosus*
(b) *S. sipylus*

![Graph showing the relationship between body size and right hind leg length for non-regenerated and regenerated legs.](image)

- Non-regenerated leg
- Regenerated leg

Body Size (mm) vs. Right Hind Leg Length (mm)
Figure 3: Effects of regeneration on fecundity in *C. morosus* and *S. sipylus*. Regeneration reduced fecundity in *C. morosus* (wingless) but not in *S. sipylus* (winged). Over a period of 40 days, females of *C. morosus* laid fewer eggs per day than individuals not regenerating during development (1-Factor ANOVA, F = 5.218, p = 0.0304). In *S. sipylus*, leg regeneration had no overall effects on fecundity (1-Factor ANOVA, F = 1.90, p = 0.6667).

![Graph showing effects of regeneration on fecundity](image-url)
Figure 4: Regeneration stunted wing size in *S. sipylus*. Across the range of body sizes, individuals induced to regenerate a right hind leg developed relatively smaller wings (1-Factor ANCOVA, *F* = 29.916, *p* < 0.0001)
Chapter 4:

The Costs of Regeneration: a review and framework for future research.

Abstract .......................................................................................................................... 49

Introduction .................................................................................................................. 50

Autotomy ....................................................................................................................... 52
  Benefits ....................................................................................................................... 53
  Costs .......................................................................................................................... 56

Regeneration .................................................................................................................. 58
  Performance Costs ...................................................................................................... 58
    Impaired Foraging .................................................................................................. 59
    Impaired Reproduction ......................................................................................... 59
    Impaired Survivorship ......................................................................................... 60
  Allocation Costs ........................................................................................................ 61
    Altered Development ............................................................................................ 61
    Altered Fecundity .................................................................................................. 64

Regeneration and Evolution ......................................................................................... 67
  Evolutionary Comparisons ....................................................................................... 68
  Future Directions ....................................................................................................... 71
Abstract

Many organisms have the ability to shed an appendage (autotomy) to escape a predator or fouled molting event. Despite its immediate advantage on survivorship, autotomy can have important consequences for locomotion, foraging, survivorship, and/or reproduction. Thus, regeneration is a way that animals alleviate some of the costs associated with losing an appendage. Like autotomy, however, appendage regeneration can have important consequences for a variety of aspects of fitness; in a wide range of amphibians, reptiles, fish, and arthropods, the allocation of resources to regenerate a lost appendage negatively affects somatic or reproductive growth. Previous research into the costs associated with regeneration has provided a strong framework for beginning to explore how tradeoffs associated with regeneration may have influenced its presence and/or absence in animals. However, all research to date describing the costs and benefits associated with autotomy and regeneration has been done based on a potentially misleading evolutionary comparison; the performance/fitness of individuals autotomising and regenerating an appendage is compared with that of individuals that have never lost an appendage. I suggest that for studies of the evolutionary significance of regeneration, the relevant comparison is between individuals experiencing autotomy without regeneration to individuals experiencing autotomy with regeneration. Future work in this direction promises new insights into the evolution of regenerative tendencies, as well as how regeneration may be influencing animal form and function.
Introduction

Two thousand and four hundred years ago Aristotle first noticed that lizards could shed and re-grow a lost tail. By the 18\textsuperscript{th} century, scientists were actively researching the ability of animals to shed and regenerate lost body parts. Reamur (1710 and 1712) was the first to record limb regeneration in insects and crustaceans. Trembly (1740) split hydra heads and obtained multi-headed individuals, and Bonnet (1745) cut worms into multiple pieces, each resulting in a new worm. Most noted of all, Spallanzani (1768) discovered that tadpoles could produce a new tail, salamanders could regenerate tails, legs, and/or jaws, and that slugs could even regenerate their head.

Early studies of animal regeneration encompassed three basic foci: documenting that regeneration could occur, characterizing abiotic and biotic effects on the speed of regeneration (e.g., Trembly [1740] recorded that hydra heads could regenerate faster in warmer weather and Spallanzani [1768] showed that nutrient limitation could alter rates of regeneration), and exploring how regeneration occurs at the developmental/physiological level. In 1712, Reamur suggested that small eggs existed underneath a lost leg, and once that leg was removed, these eggs would re-create the lost appendage. Pluflger (1883) suggested that food material was taken up at the wound surface and organized into the substance of a new leg. Thus, a hundred years before the theory of natural selection scientists had already begun to explore how animal development could lead to the regenerative growth of a second appendage. Regeneration remained an active focus of biological research through the end of the 1800's and into the
early 1900's (see Morgan, 1901; Emmel, 1905; and Dinsmore, 1996 for reviews on the early history of regeneration research).

By the 1960's, the focus of this work had shifted from an emphasis on the regenerative process itself to a broader concern for development in general. The capacity of certain animals to re-grow a lost leg or tail was exploited as a powerful tool for biologists to study fundamental aspects of development such as wound healing, blastema formation, and cell differentiation/growth. And as a result, we now understand many of the mechanistic details of the regeneration process at the genetic, cellular, tissue, organ, and organismic level. (See Goss, 1969 and Wallace, 1981 for general reviews; for taxon-specific reviews, see: lizards: Belairs and Bryant, 1985; amphibians: Scadding 1977 and 1980, Tsonis, 1991; Brockes, 1997 and Nye et al, 2003; fish: Wagner and Misof, 1992; Becerra et al., 1996; crustaceans: Bliss, 1960, Skinner, 1985 and Juanes and Smith, 1995; Hopkins, 2001; echinoderms: Dubois and Ameye, 2001; insects: Needham, 1965; Bulliere and Bulliere, 1985; spiders: Vollrath, 1990).

Regeneration research continues today to advance our understanding of animal development. What has been secondary in this process is an interest in the morphological and ecological implications of regeneration for the organisms themselves. Why do select species retain the capacity to shed and re-grow body parts while most others do not, and what are the benefits and costs to those individuals of this phenomenon? Ironically, despite being one of the oldest of studied developmental phenomena, regeneration has not often been considered in the context of natural selection and evolution. This is
particularly striking given that numerous studies show that there can be important tradeoffs with the re-growth of an appendage. In this paper I review existing studies that address the performance and/or fitness consequences of regeneration. I focus primarily on the costs associated with this process, both because the majority of existing studies only consider costs, and because this is the area most at risk of misinterpretation. This review revealed an important and common misconception regarding regenerative costs, and I end this paper by proposing an explicit, conceptual framework for future studies of the fitness consequences of animal regeneration.

Everything from ungulate antlers and turtle shells, to bat wings and snail penises have been shown to regenerate (Bellairs and Bryant, 1985; Goss, 1987; Dytham et al, 1994). In a few taxa, regeneration can even serve as a means of asexual reproduction (e.g., earthworms and asteroids). However, the most common forms of regeneration – and those that are best characterized developmentally – all involve appendages such as legs and tails, and I focus on these for the remainder of this review. I first focus on the process of appendage loss (autotomy), and then discuss the process of secondary re-growth (regeneration).

**Autotomy**

A discussion of appendage regeneration is incomplete without first addressing the subject of autotomy. The term was originally introduced by Fredericq in 1892 (originally called ‘autotomie’), and describes the reflex severance of an appendage without aid from any source other than from the severed appendage. An example would be the voluntary
The severance of a lizard tail to distract a predator that has not yet attacked. There are three other closely related terms defined by various authors in the early 1900’s (reviewed by Wood and Wood, 1932). ‘Autopsy’ refers to a situation where an outside agent is responsible for the severance of an appendage at a pre-formed breakage plane (e.g., the loss of limb to a predator who has grasped it). ‘Autotilly’, similar to autopsy, occurs when the animal itself removes an appendage with the assistance of its mouthparts or other legs. ‘Autophage’ refers to the act of consuming a shed appendage usually, but not always, after severance from the remainder of the animal (e.g. some lizards [Judd, 1955; Grant, 1957, Clark, 1969], insects [Maginnis, personal observation], and eels [George, 1978]). Although these three additional definitions clearly distinguish the situation by which the limb is lost, ‘autotomy’ as it is used in recent literature encompasses all four definitions and is generally agreed to refer to the loss of an appendage (usually at a pre-formed breakage plane), whether reflexive or due to an outside agent (see McVean, 1975; McVean, 1982; and Wilkie, 2001 for reviews on autotomy).

Benefits of Autotomy

As hinted by the various definitions of autotomy, there are multiple benefits associated with the ability to shed an appendage. The most prevalent is predation-avoidance, which can take one of two forms depending on the species: a limb or tail may be shed after it has been clasped by a predator, or these structures may be shed preemptively as a predator approaches. Shed limbs in these latter examples can often continue to move or to release toxic substances after they have been separated, and these motile appendages can serve as an effective distraction or even as a substitute meal (moving tails of lizards:
Cooper et al, 2004; moving limbs of octopuses: Norman and Finn, 2001; substance release in sea slugs: Miller and Byrne, 2000). Many studies have shown that autotomy in the face of a predator can improve the immediate survivorship of an individual (Robinson et al, 1970; Dial and Fitzpatrick, 1983a; Daniels et al, 1986; Formanowicz, 1990), but the long-term consequences of autotomy have been less well explored. It is likely that post-autotomy survival will vary greatly depending on other species-specific methods of predator avoidance such as aposematic coloration, mimicry, claws/teeth, level of aggression, size of autotomised appendage, escape speed, etc. (Arnold, 1984; Arnold, 1985).

Survivorship benefits of autotomy as they relate to predation avoidance can take many forms. Predators and/or predation events need not be typical; spider webs, fresh sap flows, limbs that come in contact with toxins/pesticides (Moore and Tabashnik, 1989), long limbs stuck in crevasses during foraging (Norman and Finn, 2001), legs injected with spider venom (Eisner and Camazine, 1983), legs or mouthparts of parasites stuck in a host (Nuttall, 1920), tails used as burrow plugs (Arnold, 1984), crab legs harvested by fishermen (Bennett, 1973), cannibalistic attacks (George, 1978), and/or intraspecific competitions (Vitt et al, 1977; Henning, 1979) can also create situations where an individual must shed an appendage to survive. In aquatic habitats, autotomy at a pre-formed breakage plane may be especially advantageous; not only does it facilitate escape from a predator, but the pre-formed cleavage plane can speed wound healing, reduce bacterial infection, and minimize potential water-borne cues that could signal the presence of a wounded animal (Zimmer-Faust, 1989; Lawrence, 1994; Juanes and Smith,
1995). Interestingly, autotomy in the face of predation can simultaneously permit survival and facilitate predator foraging; earthworm autotomy in response to ant attacks permits the earthworm to survive and benefits the ants because the shed pieces of worm are more easily transported (Dejean et al, 1999).

Most studies that explore the benefits of autotomy concern predators (e.g., autotomy as a mechanism of escape). However, arthropods derive an additional benefit of autotomy through an entirely different form of escape: escape from a fouled molt. All arthropods have their skeleton on the outside of their body, and one inescapable consequence of an exoskeleton is that it must be periodically shed if an animal is to grow to a larger size. Molting is a complex process that involves the formation of a new and pliable exoskeleton inside the existing smaller one. When the new large skeleton is complete, the animal first climbs itself out of the old one and then expands and hardens its new one. Often, when crustaceans, insects, and spiders molt from one stage to the next, appendages become stuck in the old exoskeleton and these body parts must be shed if the organism is to survive (Robinson et al, 1991; Brock, 1999; Maginnis, personal observation). Tangled molts can be especially common in species with large, defensive forelimbs (e.g., crabs) or in species with relatively long and slender legs (e.g., stick insects). Clearly, there are multiple survival benefits that can accrue from an animals’ ability/capacity to shed a leg or tail. But these benefits do not come without costs.
Costs of Autotomy

The most obvious cost of autotomy is the impediment to efficient locomotion that results from the absence of an appendage. Loss of a leg or tail can significantly impair running, walking, gliding, balance, swimming, diving, and/or underwater propulsion (Ballinger et al., 1979; Daniels, 1985; Arnold, 1985; Punzo, 1982; Brown et al., 1995; Martin and Avery, 1998; Cooper et al., 2004). Moreover, impaired locomotor performance can translate into a decreased survivorship or the ability to forage or escape from predators (survivorship: Fox and McCoy, 2000; foraging: Smith and Hines, 1991a; Ramsay et al., 2001; Cooper, 2003; predator escape: Vitt and Cooper, 1986; Formanowicz et al., 1990; Wilson, 1992; Smith, 1995; Stoks, 1999; Downes and Shine, 2001). Autotomy may also be particularly detrimental in species where the autotomised limb functioned as predator defense; predators often prefer prey missing their defensive limbs because these individuals are easier to handle during foraging. Both turtles and birds for example, actively seek autotomised crabs during foraging (Bildenstein et al., 1989; Davenport et al., 1992).

Appendage autotomy may impair reproduction as well as survival, and this can occur in a variety of ways. First, appendage loss can affect behaviors associated with sexual selection; males missing tails may be unable to effectively defend territories, burrows, and/or females during male-male competitions (Smith, 1992; Mariappan and Balasundaram, 2003), and females missing tails may be less “attractive” to males during female choice behaviors (Martin and Salvador, 1993). Second, missing an appendage can affect social interactions; males missing a tail are at a disadvantage in species where
tails are used as social signaling badges (Fox and Rostker, 1982; Fox et al, 1990; Martin and Salvador, 1993a; Salvador et al, 1995; Althoff and Thompson, 1994). Finally, losing a tail may directly affect female fecundity. In species where the tail is a primary site of fat storage, the loss of the tail also means the loss of acquired nutrients and reserves for reproduction; tailless females lay fewer and/or smaller eggs than tailed females (Smyth, 1974; Dial and Fitzpatrick, 1981).

Another less well-documented cost associated with autotomy are its effects on behavior. Ground skinks (Scincella lateralis: Formanowicz, 1990), large Psammodromus lizards (Psammodromus algiris: Martin and Avery, 1997), Iberian rock-lizards (Lacerta monticola: Martin and Salvador, 1993a and 1993b), and damselflies (Lestes sponsa; Stoks, 1999) missing appendages were significantly less active or less aggressive than animals with all their appendages, and this reduction in behavior could likely lead to decreased foraging rates and/or mating opportunities.

In summary, the capacity to shed a body appendage has multiple, immediate advantages principally connected with permitting an individual to escape an otherwise fatal situation. However, once a limb has been lost, animals face numerous challenges resulting from the loss of locomotor or foraging abilities, loss of stored resources, or impaired social and reproductive behavior. Lost legs or tails can come at an even greater expense if either more than one limb is lost at a time (especially in animals that can autotomise up to 75% of their body mass, Ramsay et al., 2001), or if they contain additional morphological features such as glands, adhesive pads, or sensory structures used in other aspects of
behavior or physiology (Bellairs and Bryant, 1985; Norman and Finn, 2001). Costs associated with autotomy presumably vary significantly depending on which appendage is lost, the function of the appendage, and the relative significance of habitat, age class, sex, and/or condition. Many of the organisms that lose appendages however, have the ability to regenerate them.

**Regeneration**

Regenerating a lost appendage can offset many of the potential long-term costs of autotomy. Although regenerated appendages usually do not attain their full proportions (see below), they are adequate to restore some of the locomotor, foraging, reproductive and/or metabolic disadvantages the animals were facing with the lost appendage (Fox and Rotsker, 1982; Daniels, 1984; Fox et al, 1990; Martin and Salvador, 1993; Chappie and Swain, 2002). While the benefits of regeneration are obvious, the costs of re-growing a lost appendage are not. In the following section I review the costs associated with the regeneration process as they are currently discussed in the literature. Costs of regeneration can be placed into two categories: performance costs associated with relatively smaller appendages, and allocation costs associated with the physiological process of appendage regeneration.

**Performance Costs**

Regenerated appendages of lizards and amphibians (Hardy and Hardy, 1977; Daniels, 1983; Salvador et al, 1996; Fitch, 2003), crustaceans (Edwards, 1972; Savage, 1975; Elner, 1980; Weis, 1982), fish (Conant, 1970; Becerra et al, 1996; Mari-Berra, 1999),
insects (Lüscher, 1948; Wolsky, 1957; Parvin and Cook, 1968; Tanaka and Ross, 1989; Karuppanan, 1998), and spiders (Vollrath, 1990) are often smaller than non-regenerated appendages. The reduction in size can vary from slight (<5%) to extreme (~95%), and these relatively smaller limbs can impair foraging, reproduction, and/or survivorship.

Impaired Foraging

Shore crabs (*Carcinus maenas*) with regenerated chelae (the first set of appendages), for example, must choose smaller sized prey, and hence have a lower energy uptake, compared to crabs with normal chelae (Elner, 1980). The effects of smaller limbs on foraging may also be indirect, as in the case of the garden spider or the common tick. European garden spiders (*Araneus diadematus*) with regenerated legs build structurally different webs, and webs with different geometry vary in their ability to trap different prey types (Vollrath, 1987; Krink and Vollrath, 1999; Schneider and Vollrath, 1998). In the common European tick (*Ixodes ricinus*), regenerated forelimbs had altered sensory organs. These organs are used to detect carbon dioxide emitted from hosts, and it has been suggested these altered sensory organs would impair a tick’s ability to effectively locate hosts (Leonovich and Belozerov, 1992).

Impaired Reproduction

Smaller appendages specifically due to regeneration can also affect reproduction. Uetz et al. (1996) showed that mating success for male brush legged wolf spiders (*Schizoeosa ocreata*) with regenerated legs was significantly lower than for males with non-
regenerated legs; regenerated legs lacked tufts of hairs used in courtship behaviors, and as a result these males were less attractive to females. Male large Psammodromus lizards (Psammodromus algirus) with regenerated tails had proportionately smaller home ranges, and hence reduced access to females, when compared with males with normal tails (Salvador et al, 1995; Salvador et al, 1996). And in shore crabs (Carcinus maenas), regenerated chelae (used to grasp the female during mating) significantly reduced reproduction in medium sized males (interestingly, relatively small and large males experienced only minor reductions in mating success; Sekkelsten, 1988). The effects of limb regeneration on reproduction can even be manifested through mate calling/singing. In bush crickets (Ephippiger ephippiger), regeneration of a leg can produce proportionately smaller hearing organs on the femur or tibia, and females with regenerated legs are less likely to respond to calling/singing males (Lakes and Miiche, 1989).

Impaired survivorship

Regeneration of a lost appendage can also affect survivorship. Wilson (1992) and Fox and McCoy (2000), have shown decreased survivorship in side-blotched lizards (Uta stansburiana) regenerating tails based on mark-recapture studies. In studies that have looked at percentages of autotomised and regenerated appendages in natural populations, many demonstrate a significantly lower number of regenerating individuals versus the number of individuals experiencing autotomy (Savage, 1975; McVean and Findlay, 1979; Liu and Wang, 1999; Lysenko et al, 2000). Although never explicitly tested, the decreased rate of individuals regenerating compared with the observed rate of autotomy.
implies that individuals regenerating an appendage experience a reduced survivorship. However, even in studies where decreased survivorship has been unambiguously documented, the mechanism underlying the reduction in survivorship can remain unclear (e.g., fat storage depletion, reduced locomotor/escape ability, or allocation costs could all contribute to reduced survivorship). This is understandable since limbs and/or tails often serve various functions, yet it is clear that the reduced size of regenerated appendages can affect animal performance at many levels.

**Allocation Costs**

In addition to the performance costs associated with reduced appendage size, many studies clearly demonstrate that regeneration can affect somatic or reproductive growth. Re-growth of an appendage requires the allocation of resources that would otherwise have gone to somatic growth or reproduction. That is, the physiological allocation of resources organisms put into the physical re-growth of a structure, sometimes over half their total energy (Vitt et al, 1977), can translate into tradeoffs with measurable ecological consequences for these animals. Allocating energy to appendage regeneration has been shown to affect both development and reproduction.

**Altered development**

In vertebrates, regeneration may decrease overall growth rate. Juvenile Eastern fence lizards (*Sceloporus undulatus*) and bunch grass lizards (*Sceloporus scalaris*), for example, grow more slowly if they are in the process of regenerating an autotomised tail (Ballinger and Tinkle, 1979). Similar patterns can be found in the side-blotched lizard.
(Uta Stansburiana); in mark-recapture experiments, hatchlings regenerating tails grew more slowly than hatchlings not regenerating tails (Niewiarowski et al, 1997). Delayed growth could reduce fitness in two ways. First, a reduced growth rate could lead to relatively smaller adults with consequences for fecundity, status, and mating success (Ballinger and Tinkle, 1979). Second, a reduced growth rate could add to the total time required for reproductive maturation, increasing the cumulative risk of predation. Extended development could be particularly detrimental in species where early maturity or precise timing of maturity is critical for individual fitness (Ballinger and Tinkle, 1979).

In arthropods, regeneration can either accelerate or delay molting (an event necessary to commence the regeneration process). Accelerated molting permits an animal to replace it’s limb faster, but early molting can have negative consequences for overall growth and body size by truncating the time needed to accumulate resources for appendage regrowth, metamorphosis, and/or reproduction. Limb regeneration in both littoral crabs (Crytograspus angulatus, Spivak, 1990) and the common crayfish (Cambarus propinquus, Zeleny, 1905) has been shown to accelerate molting.

In other arthropods, regeneration delays molting. Prolongation of immature stages allows animals extra time to accumulate resources, potentially ameliorating at least some of the allocation costs of appendage regeneration. But delays in molting also mean the animal must survive longer without the appendage, and may increase the cumulative risk of predation. Similar to delayed growth in vertebrates, a delay in molting could have extra costs if obtaining a critical adult size or stage is crucial to some other aspect of survival.
or reproduction. Cellar spiders (*Holocnemus pluchei*, Johnson and Jakob, 1999), American lobsters (*Homarus americanus*, Emmel, 1907), and edible crabs (*Cancer pagurus*, Bennett, 1973), all delay molting in response to regeneration.

Interestingly, the effects of regeneration on growth may vary, as in the case of the freshwater crab (*Paratelphusa hydrodromous*). If a limb is lost and subsequently regenerated during the non-breeding season, growth can either speed up (to regenerate the limb faster; Devi and Adiyodi, 2000) or remain the same (to regenerate the limb at a normal rate, Suma Gupta et al, 1989). That is, during the non-breeding season energy is allocated to regeneration. Conversely, if a limb is lost and regenerated during the breeding season, animals will either not regenerate (Suma Gupta et al., 1989) or growth will be delayed until animals can acquire sufficient energy to both build up its reproductive organs and regenerate the lost limb (Devi and Adiyodi, 2000). This flexibility of allocation between somatic or reproductive growth can occur in males (testicular activity [Suma Gupta et al, 1989]) as well as females (oogenesis [Devi and Adiyodi, 2000]).

Facultative modulation of the effects of regeneration may reflect on other factors besides the breeding/non-breeding season, such as the ontogenetic stage at which appendage loss occurs or the number of appendages shed. In the American lobster (*Homarus americanus*) for example, only limbs autotomised at a certain developmental stage decreased overall growth rate (Emmel, 1907; Cheng and Change, 1993). Moreover, the effects of regeneration on growth could depend on the intensity of limb autotomy. In
edible crabs (*Cancer pagurus*, Bennett, 1973; Weis, 1982), shore crabs (*Hemigrapsus oregonensis* and *Pachygrapsus crassipes*, Kuris and Mager, 1990), and common starfish (*Asterias rubens*, Ramsay et al, 2001), severe limb loss reduced growth rate while less severe limb loss did not.

Altered Fecundity

Recent studies have also demonstrated that energy allocated to appendage regeneration can come at the expense of fecundity. Female velvet swimming crabs (*Necora puber*, Norman and Jones, 1993), plethodontid salamanders (*Batrachoseps attenuatus*, Maiorana, 1977), Texas banded geckos (*Coleonyx brevis*, Dial and Fitzpatrick, 1981), Australian skinks (*Morethia boulengeri*, Smyth, 1974), and polychaeta annelids (*Capitella sp.*, Hill et al, 1988) experienced a significant – in some cases total – loss in fecundity associated with regeneration. This reduction can be manifested through the total number of eggs, the size/mass of individual eggs, the total mass of the brood, and/or egg production and hatching times.

Although all decreases in fecundity directly reduce fitness, allocation tradeoffs between regeneration and reproduction may differ between relatively long-lived and relatively short-lived species (Maiorana, 1977; Dial and Fitzpatrick, 1981; Hill et al, 1988; Smith and Hines, 1991). In long-lived species, total lifetime fecundity is often determined through several breeding seasons. Given a long adult lifespan with multiple breeding events, the cumulative cost of remaining without a lost appendage (e.g., autotomy) may outweigh the one-time cost of regenerating an appendage; even though this re-growth
reduces fecundity in the first breeding season, animals may be able to replenish lost resources and recover full fecundity before subsequent breeding events. Regeneration experiments in the long-lived California slender lizard (*Batrachoseps attenuatus*) for example, found that animals preferentially allocated energy/resources to regeneration at the expense of reproduction (Mairoana, 1977).

In contrast, the lifetime fecundity of short-lived species tends to be determined through only a single breeding season. In these species, animals allocate all available resources to reproduction (instead of regeneration), presumably because allocation to appendage regrowth is not cost effective (i.e. the benefit of regeneration to improve future survivorship does not outweigh the cost of regeneration on lifetime fecundity).

Regeneration experiments in the short-lived side-blotched lizard (*Uta stansburiana*) and the Texas banded gecko (*Coleonyx brevis*) found that animals preferentially allocated resources towards reproduction instead of regeneration. (Dial and Fitzpatrick, 1991; Fox and McCoy, 2000).

It is worth noting that a number of studies failed to detect costs that were predicted to arise from appendage regeneration (survivorship of *Psammodromus algirus*, or *Uta stansburiana*: Niewiarowski, 1997; Althoff and Thompson, 1994; Salvador et al. 1995; feeding rates of rock lizards *Lacerta monticola*: Martin and Salvador, 1993; growth in blue king crabs *Paralithodes platypus*: Lysenko et al., 2000; growth in common starfish, *Asterias rubens*: Ramsay et al., 2001; growth in hermit crabs, *Pagurus longicarpus* or mole crabs *Emerita talpoida*: Wies, 1982). However, with some exceptions (Ballinger
and Tinkle, 1979; Andrews, 1982; Althoff and Thompson, 1994; Lawrence et al, 1986; Pomory and Lawrence, 1999), many of these experiments were performed in the laboratory and under *ad libitum* food treatments. Costs associated with the regeneration process might have been offset or alleviated by increased food uptake under these artificial conditions. That is, it is possible in *ad libitum* conditions the animal can acquire adequate resources to alleviate tradeoffs between regeneration and somatic/reproductive growth. In natural conditions, especially if a lost limb impairs behavior or foraging ability, *ad libitum* conditions are highly unlikely (Vitt and Cooper, 1986; Skinner, 1985). Ballinger and Tinkle (1979) for example, compared the effects of regeneration on body growth in the lab and in the field; in the lab under *ad libitum* conditions there was no effect, while in the field regeneration did affect body growth. Similarly, laboratory experiments of Pomory and Lawrence (1999) showed high tradeoffs between reproductive and somatic growth in an echinoderm (*Ophiocoma echinata*) only under low food levels.

In summary, regeneration, like autotomy, is associated with numerous and diverse costs. In some cases these costs can be severe, as in the California slender lizard (*Batrachoseps attenuatus*), which loses all fecundity as a consequence of tail regeneration (Maiorana, 1977). In other cases, these costs may be relatively minor or absent. Regardless, it is clear that in many species both autotomy and regeneration can be accompanied by important performance or fitness consequences. These consequences can be manifested through many aspects of development, physiology, and behavior, and can have important roles in population dynamics/biology (Harris, 1989). Multiple individuals experiencing
costs associated with the regeneration process, especially those tightly coupled with some aspect of fitness, could have large effects on the whole community (Juanes and Smith, 1985). Studies thus far that have incorporated costs associated with the regeneration process into population dynamics and ecology provide an essential background for beginning to explore the evolution of regenerative capacities in animals.

**Regeneration and Evolution**

Since the early 1960's, scientists have been interested in the evolution of regeneration, variation in regenerative tendencies, and how tradeoffs associated with autotomy/regeneration might be shaping its presence and/or absence in the animal kingdom (evolution of regeneration: Needham, 1961; Spilsbury, 1961; Barr, 1964; Juanes and Smith, 1985; Goss, 1987; Vollrath, 1990; Wagner and Misof, 1992; Carnevali and Bonasoro, 2001; evolution of autotomy: Wake and Dresner, 1967; Cooper and Vitt, 1991; and Norman and Finn, 2001). Some of the major ideas related to the evolution of regenerative abilities have been lumped into a theory called 'Goss' Paradigm' (named after Richard Goss). One of the primary concepts of 'Goss' Paradigm' is that regeneration is predicted to evolve and/or persist within populations whenever the costs of re-growing an appendage are less than the costs of remaining without the appendage. In this sense, animals faced with the unfortunate situation of having to shed an appendage have a "choice": re-grow the appendage or remain without it and allocate those developmental resources elsewhere. Goss' paradigm sets up a fitness comparison between very specific individuals: those that have lost an appendage and regenerated it, and those that have lost an appendage and not regenerated it. As I suggest in the

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following section, this is not the actual comparison made by existing studies of the evolutionary significance of regeneration. Yet, accurately evaluating how tradeoffs associated with the regeneration process have or may be influencing evolutionary patterns requires that we make the appropriate fitness comparisons.

Evolutionary Comparisons

Traditionally, studies examining the costs and benefits associated with regeneration compare individuals experiencing autotomy and regeneration to individuals not experiencing autotomy or regeneration (see “Regeneration” section above). These comparisons are very useful for addressing certain relevant questions to evolutionary biology. For example, comparing the fitness between individuals that have and have not regenerated is a powerful tool for studying tradeoffs associated with the allocation of resources to trait growth; individuals re-growing a lost leg or tail produce a major morphological structure twice, and these individuals can be compared with individuals not regenerating (e.g., producing the structure only one time) to quantify the costs associated with appendage growth. However, this comparison is not especially informative for exploring why regenerative capacities themselves evolve or persist. To examine how tradeoffs may have shaped the evolution of regenerative capacities (e.g., the evolutionary significance of regeneration), comparisons between individuals who autotomised and regenerated to individuals who autotomised and did not regenerate provides a more relevant approach. The usefulness of making comparisons in this form can best be made explicit by paralleling it to the history of studying alternate mating strategies.
Early studies of the evolutionary significance of animal alternative mating tactics were plagued by similar misleading fitness comparisons (see reviews by Austad, 1984; Dominey, 1984). Initially, scientists attempted to understand the evolution of alternate mating strategies by comparing the relative fitness of a ‘major’ male (e.g., a male who fights for reproductive success) to that of a ‘minor’ male (e.g., a male who sneaks matings). Two things were subsequently recognized. First, it was determined that in most cases, whether an individual became a major or minor male depended on unpredictable aspects of the environment and not on the inheritance of specific alleles. For example, whether male dung beetles become a ‘major’ or ‘minor’ depends on their larval environment; a male that develops with ample resources will mature into a ‘major’, while a male that develops with relatively little resources will mature into a ‘minor’ (Emlen, 2000). Second, it became clear that the mating strategies of these males were based on rules of behavior that specified how best to achieve reproductive success within each of these discrete situations. That is, if a male is in a ‘good’ situation, such as being relatively large, he will adopt a dominant tactic; in a ‘bad’ situation, such as being relatively small, he will adopt a sneaking tactic.

Upon explicit recognition of these two underlying determinants of how and why males adopt a ‘major’ or ‘minor’ tactic, new questions arose to better address the evolution of alternate mating tactics. Most important of these was: if a male encounters a ‘bad’ situation, how should it behave? Specifically, do males in this ‘bad’ situation achieve higher fitness if they perform the major/dominant behavioral tactic, or if they switch to an
alternate mating tactic? To accurately answer these questions in the light of evolution, researchers began to compare the relative fitnesses of animals in a ‘bad’ situation that guard, to animals in that same ‘bad’ situation that sneak. Once this subtle shift in perspective had been recognized and implemented, the field literally exploded with informative empirical and theoretical research. We now understand many of the selective situations that have shaped the evolution of alternative mating tactics through comparisons between two males in ‘bad’ situations, each employing its own discrete tactic.

This same logic can be applied to studies of the evolutionary significance of appendage regeneration. Individuals regenerating lost appendages are presumably making the best of a bad situation, having had to shed an appendage to survive a predation or a fouled molting event. Similar to alternate mating tactics, unpredictable environmental conditions determine whether or not individuals lose an appendage and end up in a ‘bad’ situation. Comparing the relative performance/fitness of individuals in this unfortunate situation with that of individuals in a very different situation (e.g., those that have not lost an appendage) is just as misleading as comparing the fitnesses of ‘major’ males that guard to that of ‘minor’ males that sneak. To address the evolutionary significance of regeneration, the appropriate question is, given that an individual has lost an appendage, would that individual achieve a higher fitness, on average, if it allocated resources towards regeneration? Or would it do better if it did not expend resources to appendage re-growth and instead remained without the appendage? Consequently, effective exploration of the evolutionary significance of regenerative capacities requires a
comparison between animals that autotomise and regenerate and animals that autotomise and do not regenerate (Figure 1).

Studies making comparisons in this form would provide much greater insight as to if, and how, the costs of regeneration may have shaped its presence and/or absence in the animal kingdom. However, such studies are, to my knowledge, absent. One reason for this is that the biologically relevant variation appears to be rare in natural populations: species that autotomise tend to be fixed for their tendency to regenerate. The presumption here is that regeneration is always adaptive, and therefore why it is fixed in the population. Studies that compare individuals that have and have not regenerated attempt to test this presumption, but I suggest that while this may be more feasible, it is not appropriate. Comparing individuals that experienced just autotomy to those experiencing autotomy and regeneration would allow for a much more relevant fitness comparison, and thus effectively test the presumption that regeneration is adaptive. The fact that many species are fixed on their ability to (or not) regenerate, merely highlights the need for careful choice of focal species and for novel (e.g., perturbation) experimental approaches in future work.

Future Directions

In this final section, I propose research approaches that capitalize on this refined fitness comparison that promises greater resolution to explorations of how the costs and benefits of appendage regeneration may be shaping its evolution. These all involve empirical
methods that generate the requisite variation among individuals within a species, or that capitalize on such variation as it occurs among species.

First, we could carefully manipulate the timing of autotomy and take advantage of the fact that regeneration takes time. In a captive setting, it is possible to rear two groups of individuals: one group that experienced autotomy early in their development and completed the regeneration process, and another group that only recently autotomised and did not have time to allocate resources into regeneration. The performance of relevant behaviors of the recently autotomised individuals could then be compared with that of the same-age individuals that already completed the regeneration process. This is perhaps most feasible in species that naturally shed appendages with minimal provocation (e.g., lizards), and could prove most insightful for our understanding of the costs and benefits of regeneration as they relate to locomotion, foraging, and behaviors associated with reproduction. That is, this method would be most useful for behavior or physiology measures that can be measured over a brief period, instead of fitness measures such as fecundity and survivorship that are often determined through lifetime success.

Second, it is possible to engineer the missing phenotype. Again, many species with the ability to regenerate generally do so all of the time. Instead of allowing regeneration early in development, we could prevent the regeneration process to create the relevant comparison. Simple cauterizing techniques, or perhaps new hormonal/genetic techniques, could be applied to species that always regenerate after autotomy, and hence prevent the regeneration process. This technique would be especially feasible in species
that autotomise at a pre-formed breakage plane and/or where the rate of regeneration is relatively fast, and would permit comparisons between individuals that experience autotomy and regeneration to those prevented from regenerating. In addition, it would allow for comparisons at a range of different developmental stages. That is, we could explore whether the costs of regeneration (compared to the costs of just autotomy) differ based on age and/or maturity; juveniles, non-breeding adults, and breeding adults might experience different costs from the same developmental phenomenon.

Finally, we could approach the problem from a phylogenetic perspective. Many groups of lizards and spiders, for example, have clades within which there are some species that only autotomise, and others that autotomise and then regenerate (e.g., *Agama* lizards: Arnold, 1984). We can use the manipulation experiments described above to compare the relative costs and benefits of the two types of individuals, then look at whether the costs outweigh the benefits (or vice versa) in the predicted direction. In addition, we could look for correlations between gains and losses of the tendency to regenerate within specific ecological or social/behavioral conditions, and try to identify consistent circumstances that are associated with tendency to (or not) regenerate. Several species of spiders for example (*Leiobunum nigripes, Holochemus pluchei*), are capable of autotomy but not regeneration. Missing appendages in these species has no apparent performance cost in situations relating to foraging, mating and/or survivorship (Guffey, 1998; Johnson and Jakob, 1999; Dodson and Schwaab, 2001). Although this scenario makes evolutionary sense (e.g., if there is no performance cost to missing the appendage then selection should not favor the potentially costly allocation of resources to regenerate...
it), we currently do not have research to support this idea. Both testing the predictions about tradeoffs and looking for correlations within clades (such as lizards or spiders) could offer further insight into how to conduct appropriate comparisons and the selective pressures on the tendency to regenerate after autotomy.

Perhaps the best approach to this problem will involve further exploration into those species that are naturally variable in their tendency to regenerate. Some crustaceans, for example (see pages 63-64), will only regenerate lost limbs outside of the breeding season or at certain developmental stages. Some insects show the same pattern; mantids (Karuppanan, 1998) and true bugs (Lüscher, 1948) for example, are only capable of regenerating limbs if they are lost early in development. Similar to the approaches described above, careful manipulation of the timing of autotomy and regeneration would allow natural comparisons of individuals experiencing autotomy without regeneration to those experiencing autotomy with regeneration. Although all known facultative cases exist in arthropods, researchers have not specifically looked for this variation in other taxa, and it is possible that it occurs in vertebrates as well.

It will be critical for modern researchers to look for variation in the expression of regeneration. Although it is clear that a breadth of taxa regularly shed and re-grow body parts (see Table 1), it will be the subset of species that vary in their regenerative tendencies that are likely to be the most promising for empirical studies of the evolution of regeneration. A combination of phenotype engineering, laboratory experiments, phylogenetic comparisons, and further sampling of natural populations provides ideal
opportunities for researchers to disentangle the costs and benefits of autotomy, and perhaps more importantly, the costs and benefits of regeneration. Although these comparisons are not perfect, they come much closer to a meaningful fitness comparison than the existing comparisons between regenerated and non-autotomised/non-regenerated individuals.

Exploring when and where regenerative tendencies have evolved, and why, promises to bring studies of this phenomenon full circle. Exciting advances await researchers able to explore the evolution of regeneration in its natural context. It is likely that a myriad of factors influence the tradeoffs, and hence the selective pressures, associated with autotomy and regeneration. Some of these potential factors include foraging methods, locomotion, habitat type, predator and prey densities, appendage function, extent of autotomy, pattern and speed of regeneration, phylogeny, general life history traits, and the condition (health/age/sex) of the animal. Including these factors into comparisons on an evolutionary level will allow us to specifically look at how regeneration, one of the most primitive developmental processes, has shaped animal form and function. Asking the appropriate evolutionary questions will be essential for advancing our understanding of the evolution of regenerative tendencies, and future work will hopefully provide generalizations about what the costs of regeneration are and how they affect fitness.
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**CLASS ARACHNIDA**

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**Heterophrynus clabapas**

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**Maleville and De Regg, 1981**

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Figure 1: Regeneration research compared with alternate mating tactics. Research into the tradeoffs associated with regeneration can be paralleled to the tradeoffs associated with alternate mating tactics. The benefit of switching to an alternate mating tactic is only made clear through the comparison between two males in a ‘bad’ situation; a small, sneaking male has a higher relative fitness than a small, guarding male (a difference of “b” in figure A). For regeneration tradeoffs, the comparison between an animal in a ‘good’ situation (no autotomy and no regeneration) and a ‘bad’ situation (autotomy and regeneration), yields a difference in relative fitness of “a” – the perceived “cost” of regeneration in current literature. A more relevant evolutionary comparison between two animals in ‘bad’ situations, both autotomising but only one regenerating, yields a difference in relative fitness of “b”. This relative difference in fitness can be positive (see “b” in figure B), and hence a benefit of regeneration, or it can be negative (see “b” in figure C), and hence a true cost of regeneration.
Current comparisons of regeneration "costs":

More relevant comparisons of regeneration "costs":

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Chapter 5:
Field research with *Didymuria violescens*, the spur legged phasmid

Abstract

Experiments and observations on a natural population of a winged Australian phasmid provided details of many aspects of autotomy, regeneration, and flight and mating behavior as they occur in the wild. In *D. violescens*, leg loss was found to be common during all stages of development, and front legs were lost more often than middle or hind legs. Rates of autotomy and regeneration ranged from ~10% in nymphs to ~25% in adults. Autotomy in this population was due to both complications with molting and predation, and regeneration affected survivorship. In addition, the primary function of wings is to control the trajectory and speed of free-fall descents. The data gathered from field research on *D. violescens* provide a natural context for interpreting the costs associated with the regeneration process.

Introduction

Discussing the fitness consequences of leg regeneration begs for information on how often regeneration occurs in nature. In particular, determining the rates of regeneration in natural settings offers insight about how often animals experience tradeoffs associated with regeneration, and if and how these tradeoffs have affected their evolution. The three goals of this field study were to 1) sample a population for leg loss and regeneration from eclosion (e.g., hatching) to maturity, 2) determine the causes of leg loss in natural
populations, and 3) determine the primary function of wings, a trait known to be negatively impacted by leg regeneration (see Chapter 2).

Sampling a population of stick insects from eclosion to maturity, instead of just sampling adults, answers a variety of questions. First, are some developmental stages or legs more susceptible to autotomy than others? In many arthropods, limb loss is more likely in earlier developmental stages (Hardy and Hardy, 1977; Bulliere and Bulliere, 1985; Karuppanan, 1998), and forelimbs and hind limbs are lost more often than mid limbs (Lüscher, 1948; Edwards, 1972). And second, do individuals regenerating limbs demonstrate decreased survivorship? In mark recapture studies, Fox and McCoy (2000) showed decreased survivorship in lizards regenerating tails. Furthermore, in studies that have surveyed natural populations for autotomy and regeneration, many demonstrate a lower percent of animals with regenerated appendages compared to the high proportion of individuals that are missing appendages; these patterns suggest decreased survivorship in animals with missing and/or regenerating legs (Savage, 1975; McVean and Findlay, 1979; Liu and Wang, 1999; Lysenko et al, 2000). Sampling individuals at every stage will allow for assessments of if regeneration affects survivorship.

An additional goal of this field research was to determine the primary cause of leg loss in natural populations. There are two non-mutually exclusive hypotheses. First, limbs are lost primarily through predation attempts; if a predator were to grab a leg instead of the body, the animal can shed the leg and drop to the ground to escape predation (Brock, 1999). And second, limbs are lost primarily through complications with molting. Stick
insects molt upside down with elaborate twisting and turning maneuvers to separate themselves from their old exoskeleton. During these molting events, legs can become stuck in the old exoskeleton and must be shed if the animal is to survive. In the laboratory, legs are lost to molting events approximately 30% of the time (see Chapter 3), but this proportion may or may not be representative of natural populations.

Finally, I aimed to determine the primary function of stick insect wings to accurately design flight experiments in the laboratory. There are four hypotheses to explain how and why stick insects use their wings in natural habitats. First, they might use their wings to disperse for food, mates, and/or oviposition sites. Second, they may flash their wings to attract mates. In some winged species, especially in those where only males have wings, the males have brightly colored wings, which may be used during mating displays. Third, wings may facilitate escape from predators. Although this might seem an obvious use for insect wings, other natural history accounts suggest walking sticks typically drop to the ground, rather than fly, to escape predation (Carlberg, 1984; Dudley, personal communication). And fourth, if stick insects do feign death and drop to the ground to escape predation, they might use their wings to slow and control a free-fall descent from the canopy.

Study System

*Didymuria violescens* (Leach) is native to south-eastern Australia. Both sexes look similar as nymphs, but differ in overall appearance as adults. Males are fully winged, dark purple in color, and have enlarged femora bearing three or four large spines; females
are partially winged, green, lack enlarged femora and spines, and are thicker throughout
the thorax and abdomen (Figure 1). Adults become sexually mature within one month of
their final molt, and mate for extended periods of time (Figure 2). Females simply drop
their eggs to the ground from the canopy (usually in February), and the eggs incubate in
the leaf litter for 18-20 months (Neuman, 1977). The eggs hatch the following year when
the temperature rises (usually October through November). Rare among phasmids, most
eggs are from mated females, and parthenogenesis only occurs 0.6% of the time
(Campbell and Hadlington, 1967). The nymphs undergo 6 instars, and take
approximately three months to mature. *D. violescens* feeds on a variety of eucalyptus
species (particularly *Eucalyptus radiata*, *E. robersoni*, *E. dives*, *E. viminalis*, and *E.
regnans*; Neuman, 1977). Although common in many areas of New South Wales and
Victoria, it only reaches outbreak densities in higher elevations (this species was chosen
because of its pest status; finding decent sample sizes of a such a cryptic insect is not
trivial.). *D. violescens* outbreaks were first recorded in 1960/1961, and since then they
have been estimated to destroy 40% of the *E. regnans* forests (Neuman, 1977). Until the
mid-1980’s, the government regularly applied pesticides to the area, including cyanide
and DDT (Neuman, 1977).

Methods

1. When does autotomy and regeneration occur?

An outbreak population of *Didymuria violescens* was located in Bago State Forest, New
South Wales. A survey of the area determined the outbreak to be approximately 50
meters by 120 meters. Within this area, we set up 5 transects, each one 120 meters long.
and 8 meters apart. We walked each transect looking for stick insects and upon locating one, we measured its body length (to determine instar, Table 1) and visually inspected it for missing and/or regenerated legs (Figure 3). This process was non-lethal, and after measurements and inspection, animals were returned to the tree. Since the sexes aren’t distinguishable until the 3rd instar, rates of regeneration for males and females were combined until maturity. After all transects were completed (approximately 7-10 days), we did not re-sample the area for another 5 days. This waiting period was sufficient to allow them to molt to the subsequent instar, and minimized the chance we measured the same individual in the same instar twice; since individuals do not develop functional wings until they mature, the nymphs do not move large distances and were not extremely mobile relative to the sampling area. The population was sampled during all instars except the fifth. By the fifth instar it was possible to have completely regenerated legs that were only slightly shorter than non-regenerated legs, precluding scoring of regenerated individuals in the field.

Table 1: Body lengths of instars of *D. violescens*. Adapted from Neuman (1977).

<table>
<thead>
<tr>
<th>Instar</th>
<th>Average Body length (cm)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.80</td>
<td>.5</td>
</tr>
<tr>
<td>2</td>
<td>2.88</td>
<td>.5</td>
</tr>
<tr>
<td>3</td>
<td>3.89</td>
<td>.6</td>
</tr>
<tr>
<td>4</td>
<td>5.74</td>
<td>.8</td>
</tr>
<tr>
<td>5</td>
<td>7.67</td>
<td>.8</td>
</tr>
<tr>
<td>6</td>
<td>8.68</td>
<td>.8</td>
</tr>
</tbody>
</table>
In addition to the data collected on the 1st – 4th instar nymphs, 803 adults (411 males, 392 females) were collected and brought back to the laboratory. We measured their body size and all six leg lengths. Because regenerated legs are always smaller than non-regenerated legs (Ramme, 1931; see Chapter 3), paired t-tests between opposite legs (e.g., both front legs, both middle legs, and both hind legs) were used to identify regenerated legs. To determine if certain legs were lost more frequently than others (e.g., front legs, middle legs or hind legs), I used a Chi-Squared test (null hypothesis was equal frequencies of leg loss across all three pairs of legs). To test for differences in autotomy between developmental stages (e.g., 1st, 2nd, 3rd, 4th and 6th instar), I estimated the ‘expected’ value of autotomy by averaging the rate of leg loss during an instar; the average rate of leg loss per instar was 11.4% (7.7% in first, 13.2% in second, 11.2% in third, 9.6% in fourth, and 15.3% in sixth, see Table 2). Thus, my null hypothesis was that every instar should result in another 11.4% of the population losing a leg. To determine if autotomy/regeneration affected survivorship, I also used a Chi-Squared test with 11.4% as the expected value of regeneration in each stage. Since individuals always regenerate lost legs, the proportion of individuals missing a leg can also be used to predict the proportion of individuals that should be observed to be regenerating a leg. Individuals cannot physiologically commence regeneration until the third instar, so expected rates of regeneration were: 22.8% of 3rd instar nymphs (11.4% from 1st instar + 11.4% from 2nd), 34.2% of 4th instar nymphs (22.8% from 3rd instar + 11.4%), and 45.6% of adults (34.2% from 4th instars + 11.4% of 5th instars; individuals that lose a leg during the 5th instar often don’t regenerate them).
II: Why do they autotomise?

To address whether limbs are lost primarily to complications with molting or to predation attempts, we created predator-free environments (it is impossible to control molting complications in natural environments). Ten trees, all approximately 20 feet high, 3 inches in dbh, and 4-foot canopies, were covered in mosquito netting (Figure 4). Before covering the tree, we manually removed all visible insects and then placed 25 first instar nymphs in each tree (for a total of 250 individuals). The sexes were indistinguishable at this time, so no effort was made to perform the experiment with an equal number of males and females. Individuals were kept contained until maturity, at which time they were measured and inspected for leg loss and regeneration.

III: What is the primary function of wings?

To discriminate among the four hypotheses for stick insect flight, I performed three experiments/observations. First, I imitated predation attempts by approaching individuals with a stuffed bird or a human hand (n = 150 attempts; 75 bird [37 male, 38 female], 75 hand [38 male, 37 female]). I used a t-test assuming unequal variances to test for differences between the sexes; males and females may use different escape behaviors, since males are fully winged and females are only partially winged. Second, I set up 50 mating pairs to observe pre- and post-copulatory mating behaviors; males of *D. violescens* have bright purple wings that could be used for flash displays. Males and females were gathered from the trees and placed in individual mosquito netting cages (only one male and one female were in the cage at any given time). Pairs were observed for 30 minutes and then released. In addition, I observed 188 naturally occurring mating
pairs for copulatory behavior. And third, I collected and examined aerial photographs and maps from Bago State Forest in New South Wales; although individuals do not use their wings to disperse for oviposition sites or mates (observations on adults revealed a fairly equal sex ratio and females simply dropping their eggs to the ground), they may disperse for food. Although this method of determining the purpose of stick insect wings is not experimental, if outbreaks were scattered across the landscape over time, it would suggest that they might regularly disperse to find new food sources. This is likely considering they may quickly deplete food resources in outbreak densities.

Results

I: When do they autotomise and regenerate?

*D. violescens* lost legs during all stages of development (Table 2). Approximately 8% of first instar nymphs and 13% of second instar nymphs were missing legs. Individuals were unable to commence the regeneration process until the third instar. For third instar nymphs, ~11% were missing legs and ~10% had begun the regeneration process. For fourth instar nymphs, ~10% were missing legs and ~12% were in the process of regenerating. Approximately 25% of all sampled adults regenerated at least one leg during development (24.2% of males [n = 411]; 25.6% of females [n = 392], Figure 5). In addition, 5.7% of all adults regenerated two legs during development, and 3.2% regenerated three or more (n = 803).
Table 2: Frequencies of autotomy and regeneration in a population of *D. violescens*

<table>
<thead>
<tr>
<th>Instar</th>
<th>N</th>
<th>% of individuals missing at least one leg</th>
<th>% of individuals in the first stage of regeneration</th>
<th>% of individuals in the second stage of regeneration</th>
<th>% of individuals with complete regenerated legs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>482</td>
<td>7.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>502</td>
<td>13.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>509</td>
<td>11.2</td>
<td>10.2</td>
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<td>-</td>
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<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>519</td>
<td>9.6</td>
<td>8.7</td>
<td>3.2</td>
<td>-</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>803</td>
<td>15.3</td>
<td>&lt;.0</td>
<td>9.4</td>
<td>14.8</td>
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Based on the data gathered on both the nymphs and adults, front legs were lost and regenerated more often than either mid legs or hind legs ($\chi^2 = 40.01, p < 0.001$, Table 3). There were no significant differences in the rate of autotomy between different developmental stages ($\chi^2 = 8.68, p = .05$). The 1<sup>st</sup> through 4<sup>th</sup> instars lost legs at a similar rate.

Table 3: Proportions of missing or regenerated legs that were front legs, mid legs, or hind legs (males and females combined).

<table>
<thead>
<tr>
<th>Instar</th>
<th>% of missing or regenerated legs that were FRONT LEGS</th>
<th>% of missing or regenerated legs that were MID LEGS</th>
<th>% of missing or regenerated legs that were HIND LEGS</th>
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<tr>
<td>1</td>
<td>51.4</td>
<td>21.6</td>
<td>27.0</td>
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<tr>
<td>2</td>
<td>42.4</td>
<td>28.8</td>
<td>28.8</td>
</tr>
<tr>
<td>3</td>
<td>50.5</td>
<td>19.2</td>
<td>30.3</td>
</tr>
<tr>
<td>4</td>
<td>49.6</td>
<td>18.9</td>
<td>31.5</td>
</tr>
<tr>
<td>6</td>
<td>41.3</td>
<td>33.2</td>
<td>25.5</td>
</tr>
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</table>
Leg regeneration also affected survivorship. Based on how often nymphs lose legs, I expected to see at least 46% of the adults in this population with evidence of having lost or regenerated leg. Sampling revealed approximately 25% ($\chi^2 = 1597.35, p < 0.001$).

II: Why do they autotomise?

In the predator-exclusion experiments, 17.8% of adult males ($n = 76$), and 11.9% of adult females ($n = 42$) regenerated one leg during development. These rates of regeneration were lower than the rates in the free-living population. Four out of the ten trees were destroyed by cows ($n = 100$); 29 individuals died; 3 individuals were unaccounted for ($n = 250$).

III: What is the primary function of wings?

When presented with a stuffed bird, 16.0% of individuals remained motionless and 84.0% dropped to the ground ($n = 75$ encounters, Table 4). When approached by a human hand, 18.6% of individuals remained motionless and 81.4% of individuals dropped to the ground ($n = 75$ encounters, Table 4). There were no significant differences between the behaviors of males (fully winged) and females (partially winged; $p = .006$, Table 4), and no animals used their wings to escape.

Out of the 50 pairs of males and females put in mesh cages, 21 begun mating within 30 minutes. None of these males displayed his wings to the female prior to copulation. Out of 188 observed mating pairs in their natural habitat, 7.4% ($n = 14$) of the males had their wings out at one point during copulation.
Aerial photographs and maps of *D. violescens* outbreaks in Bago State Forest since the early 1980’s reveal a slow yet steady progression of populations. Out of nearly 40 outbreaks in or near Bago State Forest since 1981 (n = 36), only 25% (n = 9) were not adjacent to a previous years’ outbreak. Although not as informative as a mark-recapture study, this information does suggest that this species does not regularly travel far distances, but tends to remain within a local area.

Table 4: Summary of results from predation experiments on *D. violescens*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% remained motionless</th>
<th>% dropped to the ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males approached with stuffed bird <em>n = 37</em></td>
<td>13.5</td>
<td>86.5</td>
</tr>
<tr>
<td>Females approached with stuffed bird <em>n = 38</em></td>
<td>18.4</td>
<td>81.6</td>
</tr>
<tr>
<td>Combined ‘bird’ results <em>n = 75</em></td>
<td>16.0</td>
<td>84.0</td>
</tr>
<tr>
<td>Males approached with human hand <em>n = 38</em></td>
<td>23.7</td>
<td>76.3</td>
</tr>
<tr>
<td>Females approached with human hand <em>n = 37</em></td>
<td>21.6</td>
<td>78.4</td>
</tr>
<tr>
<td>Combined ‘hand’ results <em>n = 75</em></td>
<td>22.6</td>
<td>77.4</td>
</tr>
</tbody>
</table>

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Discussion

I: When do they autotomise and regenerate?

Results from this study suggest that *D. violescens* loses legs during all stages of development (even eclosion), and that regeneration is not visible until the third instar. It is worth noting that this is consistent with laboratory findings in *Sipyloidea sipylus* and *Carausoius mororsus*; in captive settings, individuals that lost legs early in development showed no visible signs of regeneration until the third instar. Front legs were lost more frequently than either middle legs or hind legs, and there appear to be no differences in autotomy and regeneration rates between males and females.

Regeneration affected survivorship. Based on how often nymphs lose legs, I expected to find nearly half of all adults with partially or completely regenerated legs; sampling revealed only a quarter. Decreased foraging ability and/or impaired locomotion probably contributed to decreases in survivorship.

II: Why do they autotomise?

The data gathered from adult *D. violescens* and the predator exclusion experiments suggests both predation and complications with molting play important roles in autotomy; rates of leg regeneration in the enclosed trees were nearly half of the observed rate in natural populations. This suggests that perhaps ~10-15% of all phasmids experience complications with molting at some point during their lifetime, and predation attempts add to that rate of autotomy. It is possible, however, that other atypical predation attempts took place within the enclosed trees. On many occasions we saw legs
stuck in spider webs or fresh sap flows (Figure 6); it is quite possible sap flows and
spider webs contributed to leg loss within the enclosed trees. It is also worth noting that
individuals escaped predation attempts on structures besides the legs; several dozen
adults were found missing abdomens that appeared to have been lost to the grasp of a bird
(Figure 7).

III: What is the primary function of wings?

Based on the two experiments and mating observations, *D. violescens* primarily use their
wings to slow and control their fall from an elevated position. Although approximately
7% of males had their wings out during some point of copulation, the amount of time
their wings were spread was extremely short and made during adjustments in copulation;
it appeared to be for balance rather than courtship. Moreover, inspection of aerial
photographs revealed most outbreaks occurred adjacent to the previous years’ outbreak.
Combined with the observation that dispersal is not needed for mate location and/or
oviposition this information suggests that this species does not regularly travel long
distances. The need to travel, however, likely varies depending on environment; other
species with different sex ratios and/or habitat structures may use their wings for
dispersal to a greater extent.

The predation experiments revealed a strong tendency for *D. violescens* to drop to the
ground to escape predation. Both strong winds and flocks of cockatoos frequently forced
individuals out of the canopy, and both males and females used their wings to slow and
control their descent. It is worth nothing that both males and females tended to drop to
the ground when presented with a predator, despite a large difference in relative wing size. Males are fully winged and females are partially winged, suggesting that perhaps females would not drop to the ground as easily and males. That is, flight in the partially winged females may be poor, and hence these animals might have a stronger tendency to remain motionless in the face of predation. The result that both fully and partially winged stick insects have similar behaviors in the face of predation suggests using wings to control a descent is common and useful for all wing types. This information was used to accurately design flight experiments in the laboratory (see Chapter 2).

Conclusion

To my knowledge, this is the first study of stick insects in their natural habitat. Observations and experiments during all stages of development revealed patterns about their autotomy and regeneration habits, as well as insight to their behaviors relating to mating or flight. More field research into other species will reveal if the rates of regeneration and usage of wings shown in D. violescens is typical of other phasmids.
Figure 1: Male (left) and female (right) adult *D. violescens*.
Figure 2: Male and female *D. violescens* in copulation.
Figure 3: Missing legs and the first two stages of regenerated legs are easily identifiable in the field. (a) shows a missing leg, (b), (c), and (d) show the three stages of limb regeneration; the first molt after limb loss produces a leg about $\frac{1}{4}$ the normal size (b), the second molt produces a limb about $\frac{1}{2}$ the normal size (c), and the third molt produces a leg nearly normal in length (d).
Figure 4: Examples of predator-free trees in Bago State Forest.
Figure 5: Frequencies of leg loss and regeneration in adult *D. violescens*. Out of all collected adults, 15.3% were missing legs, and 25.2% had either partially or completely regenerated legs. All six legs were found to be missing or regenerated; the percents below indicate the proportion of all adults missing or had regenerated that specific leg.

<table>
<thead>
<tr>
<th>Leg</th>
<th>Missing (%)</th>
<th>Regenerated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front Left</td>
<td>2.7%</td>
<td>6.2%</td>
</tr>
<tr>
<td>Front Right</td>
<td>1.8%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Mid Left</td>
<td>3.0%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Mid Right</td>
<td>3.2%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Hind Left</td>
<td>2.4%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Hind Right</td>
<td>2.2%</td>
<td>3.1%</td>
</tr>
</tbody>
</table>
Figure 6: *D. violescens* leg lost to a spider web.
Figure 7: Male *D. violescens* missing part of his abdomen, presumably due to a predator.
Appendix 1:

Supplemental Results on Flight Experiments with *Sipyloidea sipylus*

Leg regeneration induced reductions in wing area negatively affected three additional measures of flight performance in addition to time aloft and the proportion of crash landings: distance traveled, their relative flight ‘control’, and/or the direction of their descent.

Distance and/or ‘control’ may be ecologically relevant consequences of stunted wings. How far an individual can travel during a descent may be important in relatively open habitats, where getting from one tree to another is necessary for mate location and/or efficient foraging. In addition, the control of a descent may also be relevant to risk of predation and injury; a relatively uncontrolled descent could be a potential cue to predators and/or increase the risk of striking a branch during their fall. Stick insects have three behaviors they can perform during a free-fall: thanatosis (feigning death and dropping quickly to the ground), gliding, or flapping. Stick insects in thanatosis have some control over their descent; individuals gliding have relatively more control over their descent; individuals flapping have the most control over their descents. Thus, the amount of time spent gliding and/or flapping (compared to thanatosis) can be used as an index of flight control and performance.

Directional descents are considered to be an important intermediate stage in the evolution of insect flight (Yanoviak et al., 2005). Some tribes of ants, for example, also forage
high in the canopy and are regularly forced off of leaves or branches by strong winds and/or vertebrates. Although they lack wings, the ants have adapted to falls from the canopy by directed aerial descents that follow a ‘J’ shaped path to return to the trunk of the tree they fell from (Dudley, *personal communication*). Their directed descent follows three stages: a vertical drop, a directional adjustment and body alignment, and a steep directed glide towards the tree trunk (Yanoviak et al., 2005). When forced out of a tree branch by strong winds, some stick insects followed a similar pattern (Maginnis, *personal observation*). Examination of the direction of descent with *S. sipylus* revealed if this insect is using similar techniques, and if relatively smaller wings negatively affects this behavior.

**Methods**

**I. Distance**

Upon thanatosis (feigning death), individuals were held by the thorax, dorsal side up, and released from a 12-foot ladder (height of release was 15 feet). To determine the distance traveled by the insect we used a 50m measuring tape. Individuals rarely moved after landing so measurement accuracy was high. Treatment groups (0 vs. 1 leg regenerated during development) were compared with an unpaired t-test assuming unequal variance.

**II. Control of Descent**

The relative control of each insect’s descent was determined through a rating system of 0 to 3, with scores based on the percentage of their total time aloft spent in thanatosis, gliding, and/or flapping (Table 1). For example, and individual who spent 40% of their
descent in thanatosis and 60% gliding received a score of ‘1’; an individual who spent 10% of their descent in thanatosis, 20% gliding, and 70% flapping received a score of ‘3’.

Treatment groups were compared with an unpaired t-test assuming unequal variance.

Table 1: Guidelines for rating individual descent performance.

<table>
<thead>
<tr>
<th>Performance Score</th>
<th>% of time aloft in thanatosis</th>
<th>% of time aloft gliding</th>
<th>% of time aloft flapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt; 75</td>
<td>&lt; 25</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 50</td>
<td>&gt; 25</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 50</td>
<td>&gt; 50</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>3</td>
<td>&lt; 25</td>
<td>&lt; 50</td>
<td>&gt; 50</td>
</tr>
</tbody>
</table>

III. Direction

The direction of descent was recorded as ‘J’ or ‘L’. ‘J’ described individuals who followed the ‘J’ shaped directional descent and glided or flew towards the ladder after thanatosis; ‘L’ described individuals who did the opposite of the ‘J’ shape and glided or flew away from the ladder. Descents with no apparent direction (e.g., individuals that flew around in a circle or spiraled towards the floor) were recorded as directionless.

Descents given a performance rating of 0 (e.g., individuals that spent at least 75% of their descent in thanatosis, see above) were not scored for direction. Treatment groups were compared with a Chi-Squared test (null hypothesis was equal frequencies of ‘J’, ‘L’, and directionless descents).
Results

I. Distance
Control individuals traveled further distances than regenerated individuals (p = .0033; Figure 1). The average distance traveled by control individuals was 2.4 m (SD ± 1.92); the average distance traveled by regenerated individuals was 1.8 m (SD ± 1.25).

II. Control of Descent
Individuals induced to regenerate performed worse in the repeated trials (p < .0001; Figure 2). The average score for control individuals was 1.75 (SD ± .87), indicating they generally came out of thanatosis within the first half of their descent and spent the other half actively gliding or flapping. The average performance score for regenerating individuals was .78 (SD ± .71), indicating very few of individuals spent time gliding or flapping.

III. Direction
*S. sipylus* did not show a tendency to follow the ‘J’ shaped pattern of direction descent. In fact, animals showed a tendency to glide or fly in the ‘L’ shaped pattern (χ² = 11.932; p = .05). Out of 250 trials (both treatment groups combined), 28.4% had a ‘J’ shaped descent, 43.6% had an ‘L’ shaped descent, and 28.0% had no direction. For control animals, 27.4% showed the ‘J’ pattern, 52.6% showed the ‘L’ pattern, and 20.0% had no direction (135 trials, n = 27 individuals, 5 trials per individual). For the regenerated animals, 29.6% showed the ‘J’ pattern, 33.0% showed the ‘L’ pattern, and 37.4% had no direction (115 trials, n = 23, 5 trials per individual). (Table 2).
Table 2: Summary of Results on Directional Descent.

<table>
<thead>
<tr>
<th></th>
<th>% trials 'J'</th>
<th>% trials 'L'</th>
<th>% trials No Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined Results</td>
<td>28.4</td>
<td>43.6</td>
<td>28.0</td>
</tr>
<tr>
<td>n = 250 trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regenerated Animals</td>
<td>29.6</td>
<td>33.0</td>
<td>37.4</td>
</tr>
<tr>
<td>n = 115 trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Animals</td>
<td>27.4</td>
<td>52.6</td>
<td>20</td>
</tr>
<tr>
<td>n = 135 trials</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

I. Distance

Although regenerated animals traveled significantly shorter distances than control animals, this result is misleading. Theoretically, an animal that flew around in a circle and then landed on the ground could go the same total distance as an individual who simply dropped to the ground. Even though the ability to travel long distances could prove useful for foraging and/or mate location, distance as it was measured in this study offers little insight as to how relatively smaller wings might affect their ability to travel in natural habitats.

II. Control of Descent

Animals that regenerated during development had less control over their descents than control animals. They were more likely to drop straight to the ground, and less likely to actively glide and/or flap. Both of these actions usually resulted in a crash landing (see page 9), and in a few cases injury (two individuals broke legs on their fifth and final
descent). While these results may in fact be a consequence of developing relatively smaller wings, whether this can be interpreted as a cost of proportionately smaller wings requires a closer look at their natural history.

In the face of predation, stick insects feign death. In fact, I took advantage of this behavior to ensure all animals began their descent in precisely the same way. Animals that received a high performance score came out of thanatosis quickly (e.g., within the first few feet of descent), spread their wings, and glided and/or flapped slowly to the ground. Animals that received low performance scores dropped for most, if not all, of the 15-foot descent. In natural habitats, this behavior may not be as costly as it was in this experiment. That is, remaining in thanatosis and dropping relatively quickly may actually be a beneficial behavior in some habitats. Stick insects regularly forage high in the canopy, sometimes well over 100 feet above the ground. Certain vegetative structures or types of predators may favor animals that feign death for at least the first 15 feet of a 100-foot drop. Experiments that involve dropping insects from heights greater than 15 feet may offer greater insight as to whether these animals feigning death are equally capable of recovering control of their descent.

III. Direction

There were no significant tendencies for S. sipylus to follow the ‘J’ shaped pattern of directional descent. In fact, there was a tendency (particularly for control animals) to do the opposite and fly/glide away from the point of release. However, since stick insects are highly arboreal and have relatively primitive wings, it is possible that experiments
performed from higher elevations and/or with partially winged or wingless species may indeed reveal a tendency for these insects to control their descents with the ‘J’ pattern. Many of my observations of the ‘J’ pattern of descent in the field for example, were made with females of *Didymuria violescens*, who are partially winged (e.g., brachypterous). Moreover, current research that is exploring how arboreal insects use controlled directional descents to survive and maneuver in forest canopies are being done from nearly 30 meters, over five times the height of release from this experiment (Yanoviak et al., 2005).

**Conclusion**

Relatively smaller wings affect other aspects of flight performance in *S. sipylus* in addition to time aloft and control of landing. Individuals regenerating legs during development travelled shorter distances, and glided/flapped for less time than control individuals. However, distance traveled may only be relevant measure of flight performance in certain habitats, and thanatosis is likely to be a beneficial, innate behavior. No significant differences or patterns were found in this species’ tendency to follow ‘J’ shaped directional descents. More research into these questions with different species, a variety of habitats, and greater heights may offer further insight as to how relatively smaller wings translates into functional costs in natural settings.
Figure 1: Distance traveled during *S. sipylus* flight. Individuals that regenerated a leg during development, and hence developed relatively smaller wings, traveled significantly shorter distances than control animals (control animals: 2.4m [S.D. ± 1.92]; regenerated animals: 1.8m [S.D. ± 1.25]).
Figure 2: *S. sipylus* flight control. Individuals regenerating a leg during development, and hence developing relatively smaller wings, spent relatively less time gliding and/or flapping during descents. Flight control was determined through a scale of 0 to 3: 0 representing relatively poor control over individual descents, 3 representing relatively high control. Animals not regenerating a leg received an average score of 1.75 (S.D. ± .87); regenerating animals received an average score of .78 (S.D. ± .71).
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124

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