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Leg Impairments Elicit Graded and Sex-specific Demographic Responses in the Tephritid Fruit Fly *Anastrepha ludens*

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Abstract

This study was concerned with the impact of different levels of artificial impairment (leg amputations) on male and female survival and female reproduction in the Mexican fruit fly, *A. ludens*. We monitored the demographic responses in a total of 100 flies of each sex that were maintained individually in $4 \times 4 \times 10$ cm and subject to 1-of-11 different leg amputations (plus intact control) including cohorts in which either one front, one middle or one rear leg was severed (3 cohorts total), in which two legs were severed in different front-middle-rear combinations (6 cohorts total), or in which the two middle and one additional leg were severed (2 cohorts total). The two main findings were that: i) although the effects on mortality of impairments were sex-specific, no universal patterns emerged that applied to either sex; and ii) reproduction occurred in all cohorts of impaired females. Moderately-impaired flies (e.g. amputation of a single middle leg) laid nearly as many eggs in their lifetime as did intact controls. However, severely impaired flies (i.e. 3 legs amputated) laid significantly fewer eggs.

Introduction

Although the concept of impairment is usually associated with humans in the context of aging, disability, and failing health, it is also relevant to the study of fitness, longevity, and survival in non-human species including insects, arachnids and other arthropods. Despite the prevalence of impairments in nature, we are aware of only a small number of impairment-related studies that elucidate the effects of injuries on the demographic (survival; reproduction) characteristics of insects or on changes in insect behavior as individuals age. These include studies on supine behavior in Mediterranean fruit flies (Carey et al., 2006; Papadopoulos et al., 2002) and the mortality consequences of injury, damage or autotomy (self-amputation) such as the effects of wing removal in the housefly, *Musca domestica* (Sohal and Buchan, 1981), leg removal (or injury) in (Carey et al., 2006; Papadopoulos et al., 2002) *Drosophila melanogaster* (Carey et al., 2007; Sepulveda et al., 2008), wing clipping in moths (Javois and Tammaru, 2004), leg impairment in juvenile wolf (Wrinn and Uetz 2008), wing-wear in fruit-feeding butterflies

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(Molleman et al., 2008; Molleman et al., 2009), and leg autotomy in spiders (Eisner and Camazine, 1983; Guffey, 1998; Johnson and Jakob, 1999; Moore and Tabashnik, 1989).

In light of the paucity of data on the influence of impairment on fitness in non-human species and the importance of understanding the dynamics of impairment more generally, the broad purpose of this study was to use the tephritid fruit fly, *Anastrepha ludens*, commonly known as the Mexican fruit fly or Mexfly, to examine the demographic response of artificial impairment resulting from leg amputation. We asked three specific questions: How do different levels or configurations of leg amputations influence mortality in each sex? How do these different impairments affect survival of males relative to females (i.e. affect the gender gap)? How do the impairments in females affect their reproductive patterns and lifetime output? This study builds on an earlier investigation {Carey, 2006 #194} concerned with the mortality consequence of impairment in *D. melanogaster*. In this study we not only examine the effects of impairment on mortality as was done with the *D. melanogaster* investigation, but also document the effects of leg removal on age-specific reproduction.

Methods

The basic conceptual framework for impairments for humans are contained in the *International Classification of Functioning, Disability and Health* (WHO, 2001) where impairments are considered a condition that causes a loss of function. Thus the amputation of one or more legs in the treatment flies in our study is considered the *cause* of the impairment but the impairment itself is the expression of the (health) condition i.e. ambulatory and/or reproductive capabilities are reduced.

Background information on both the biology of *A. ludens* and details of its mass production are given in the paper by Carey and co-workers (Carey et al., 2005). We used virgin females because the lifetime reproduction of unmated females is nearly identical to mated females in this species (Rogina et al., 2007) and replacing dead males changes the within-cage dynamics because of differences between the ages and/or mating histories of the original male and its replacement. The experimental design consisted of 12 treatments including an intact control cohort and 11 different impairment grouped into three categories: (1) *Single-leg amputations*: *Front (F)*, *Middle leg (M)*, *Rear leg (R)*; (2) *Two-leg amputations (opposite sides)*: FF, FR, FM, MM, MR, RR; and (3) *Three-leg amputations*: MMF, MMR. Legs were removed using pointed forceps.

A 1.5 μ l droplet of the full diet of yeast and sugar (Carey et al., 2005) and a 6 μ l droplet of water were supplied to individual flies each day on glass slides using separate Eppendorf® needles. Newly-emerged (virgin) individual flies were housed in 4 $\times$ 4 $\times$ 10 cm plexiglass cages, each of which was part of a 24-unit cage unit. Treatments distribution was made in a randomized block design, using different color codes to identify both the treatment and the food slides. Females and males were placed in alternate cages to eliminate the possibility of eggs from two females overlapping on the egg collection surface. Females laid eggs through organandy mesh fastened to the front of the cage and were counted daily. A total of 100 individuals of each sex were used per treatment. Daily mortality and female reproduction were monitored daily throughout the life of each fly. Environmental conditions were 12:12 LD cycle, 24.0° C ($\pm$ 2°) and 65% RH ($\pm$ 9%).

Results

Survival

To investigate gender-specific impairment influences on medfly lifespan, we applied non-parametric hazard estimation to the data grouped by treatment and gender. We observed no

difference in hazard between males and females in the control treatment group (intact), the least impaired groups (single legs amputated; i.e. F, M, R), and the most impaired groups (three legs amputated; MMR, MMF). Among the intermediately impaired groups (two legs amputated), males had larger hazard functions at FF, FR, and MM, while females have larger hazard functions at FM, MR, and RR.

The life expectancies observed for all treatments with both sexes combined are presented in Table 1 with the results of the statistical analyses presented in Table 2. Several aspects of the results contained in these tables merit comment. *First*, any level of impairment caused by leg amputation(s) reduces life expectancy. While this result was not surprising, it was conceivable *a priori* that a moderate level of impairment could have resulted in an increase in longevity through a reduction in activity as was found by Sohol and Buchan (1981) when they removed the wings of the housefly, *Musca domestica*. *Second*, the degree to which life expectancy was reduced was directly related to the severity of the impairment (i.e. effects on longevity of removal of 3 legs > 2 legs > 1 leg). For example the average life expectancies shown in Table 1 for single-, two- and three-leg amputations were 29.5, 16.9 and 11.1 days, respectively. *Third*, there were two impairment treatments that were by themselves significantly different than other impairment treatments with respect to their impact on longevity (Table 2): i) the amputation of a single middle leg which had the least impact on longevity relative to all other impairment treatments; and ii) the amputation of both middle and one front leg which had the greatest impact on longevity. That there was no statistical overlap in their effects on longevity suggests that these represent impairment thresholds for minimal (i.e. with single middle leg removed) and maximal (i.e. with both middle and one front leg removed) impact. *Fourth*, the impact on life expectancy for each amputation category (i.e. number of legs removed) was conditional on which legs or combination of legs were severed. For example, the average of the life expectancies for the cohorts in which the front, middle legs and rear were removed singly or in combination with other legs was 16.8, 21.7 and 18.4 days, respectively. In other words, removal of one or both front legs had a greater impact on mortality than the removal of one or both rear legs and removal of one or both middle legs reduced longevity less than removal of one or two other legs.

Reproduction

The normality statistical check failed for the number of eggs laid since more than half of the female medflies laid no eggs in their lifetimes. Therefore, we applied Wilcoxon rank sum test (also known as Mann-Whitney U test), to check the differences of the total number of eggs each female laid in the treatment groups. The test achieved a large χ^2 statistic at 244.3 with 11 degrees of freedom and a small p-value of less than 0.0001. Thus, leg impairments have a large negative impact on lifetime reproduction in medflies.

Although lifetime egg production by females was reduced in all treatments cohorts, the degree to which reproduction was reduced depended upon the details of the impairment treatment. For example, single leg amputations reduced lifetime reproduction by an average of only about one third relative to reproduction in intact flies (Table 3). In contrast, the amputation of two legs reduced egg laying by 90% relative to control flies if both front legs were amputated and by 50% relative to controls if both rear legs were amputated. Lifetime reproduction in the two treatments involving extreme impairments where both middle and either one of the front or one of the rear legs was amputated averaged only around 5% of the control reproduction. The data on both gross reproductive rates and the percent egg laying also contained in Table 3 sheds light on these differences in net rates between impairment treatments. For example, both the gross reproductive rates and the percent of females laying eggs for the single-leg amputations were similar (and in some cases non-significantly higher) to the corresponding rates for the intact control females. This suggests that the lower net reproductive rates for these treatment

flies were due to an increase in mortality resulting from the impairment and not to a reduction in individual fly egg-laying capabilities. In general amputation of one or both of the front legs had the greatest impact on lifetime reproduction whereas amputation of one or both of the middle legs had the least impact on lifetime reproduction. It is remarkable that, despite the severity of their impairment and the effects on their mobility, several of the females missing half of their legs (i.e. 3 legs amputated) still laid a small number of eggs.

Discussion

This study yielded three main results in response to the specific questions that we addressed. The answer to the first question was not surprising in that different levels and configurations of leg amputations did reduce longevity in both male and female cohorts in relation to the severity of the impairment—i.e. the greater the severity the greater the reduction in longevity. The answer to the second question that pertained to sex-specific effects of impairment also was not surprising in that we did identify treatments where the impact differed between the sexes. However, the mortality response for neither sex was consistently greater across treatments. This finding differed from the results of studies on *Drosophila melanogaster* where it was reported that injuries or impairments had a greater impact on male mortality than on female mortality (Carey et al., 2007; Sepulveda et al., 2008). The third finding was that impairment reduced the lifetime reproduction of females due primarily to a reduction in lifespan and not to any apparent disruptive effect on either the propensity or the ability of individuals to lay eggs. Although the study on *D. melanogaster* by Sepulveda et al (2008) also reported a reduction in egg laying in flies with leg injuries, they argued that the reduction was due mostly to the disruptive effect of the injury and not to the reduced lifespan of injured females. However, the differences may be because reproduction was measured for only 10 days in their study whereas it was measured throughout the life of individual females in the current study. We found that egg laying rates in moderately-impaired female flies (e.g. middle leg amputated) was only slightly reduced relative to the reproduction in intact females. We also discovered that even some of the most severely impaired females (i.e. loss of 3 legs) were still capable of laying a small number of eggs despite their impairment rendering them nearly immobile.

Our data do not support the conventional argument, known as the *risk-prone behavior hypothesis* (Owens, 2002), that because males typically engage in costly activities including male-male competition (Gerhardt, 2002), sexual advertisement (Zuk et al., 1990), and mate searching (Bell, 1990) male mortality is intrinsically higher than female mortality. All of these activities either increase the likelihood of injury or impairment. However, the life expectancy of intact male *A. ludens* in the current study as well as of medflies (*Ceratitis capitata*) in earlier studies that were maintained under a range of different conditions such as dietary restriction (Carey et al., 2008), high density (Carey et al., 1995), and starvation (Carey et al., 1999) exceeded the life expectancies of females maintained under identical laboratory conditions. Instead of a general rule on sex differentials in longevity and responses to impairments, they may be more idiosyncratic and depend on the field biology and anatomy of the species. For example, in the case of Mexflies females may suffer more from amputation of hindlegs because their center of mass is probably located nearer their caudal side given that they have a large ovipositor.

Conclusions

We have shown in a second and much larger species that higher levels of leg loss cause higher mortality and that configuration matters. These effects were sex specific, but the mortality response for neither sex was consistently greater across treatments, indicating species specific idiosyncratic effects of leg impairments. Impairments impact reproduction mainly through reducing life span in this species and even severely impaired individuals are still able to lay

some eggs. The results of this study not only strengthen the empirical foundation laid down by several previous studies concerned with the effects of injury and impairment on the life history traits in insects, but also illustrate the importance of arthropod model systems for both identifying and validating general principles concerned with the dynamics of morbidity and mortality. Consequently, we believe that disability and impairment research using arthropod models has the potential to shed light on important unresolved questions pertaining to chronic disability in the elderly (including humans) ranging from the mortality dynamics of morbidity compression to age- and sex-specific patterns of mortality resulting from conditions of comorbidity (Manton, 2008).

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Fig. 1.

Event history charts (Carey et al., 1998) for the intact control female *A. ludens* and the 11 impairment treatment cohorts. Each horizontal line corresponds to an individual female with color-coded age segments corresponding to green=0 eggs/day, yellow = 1-20 eggs/day, and red =>20 eggs/day.

Life expectancies in days (both sexes combined) for different impairments (leg amputations) of the fruit fly, *A. ludens*. F, M, and R denoted front, middle and rear legs, respectively. Life expectancy for intact control flies was 45.5 days. Because of the redundancy of treatments created by the table row-by-column configuration, some table entries are also redundant.

Table 1

Leg Amputation Right Side	Leg Amputation of Left Side				Average	Amputation of both Middle Legs
	None	F	M	R		
F	27.8	17.0	15.3	14.1	18.6	9.6
M	32.4	15.3	20.9	18.0	21.7	
R	28.2	14.1	18.0	19.2	19.8	12.5
Average	29.5	15.5	18.1	17.1	20.0	

Table 2

Grouping of treatments generated by Student-Newman-Keuls Test. Means with the same letter are not significantly different. The letters F, M and R in the treatment column denote front, middle and rear legs amputated.

Treatment	Student-Newman-Keuls Grouping		Lifespan Means
Intact		A	45.469
M		B	32.360
R		C	28.195
F		C	27.833
MM		D	20.930
RR	E	D	19.226
MR	E	D	17.995
FF	E	G	16.960
FM	H	G	15.335
FR	H	G	14.101
MMR	H		12.455
MMF	I		9.635

Table 3

Gross^a and net reproduction in the fruit fly, *A. ludens*, for 14 different impairment (leg amputation) treatments. Gross and net reproduction for intact control females were 695.2 and 312.7 eggs/female, the percent of the intact control females that laid any eggs was 70%, and the percentage of life-days in which control females laid was 24.6%. Because of the redundancy of treatments created by the table row-by-column configuration, some table entries are also redundant. (e.g. the front-mid treatment is identical to the mid-front treatment in which females laid 255.6 eggs in their lifetime).

Leg Amputation	One Side	Leg Amputation Opposite Side				Average	Amputation Both Middle Legs ^b
		None	Front	Mid	Rear		
GROSS	Front	723.6	80.1	255.6	151.6	302.7	38.6
	Mid	578.2	255.6	455.1	495.2	446.0	
	Rear	866.9	151.6	495.2	578.1	523.0	39.0
	Average	722.9	162.4	355.4	408.3	412.3	38.8
NET	Front	213.8	35.0	35.9	82.6	91.8	8.0
	Mid	234.8	35.9	146.5	58.0	118.8	
	Rear	236.1	82.6	58.0	163.8	135.1	20.4
	Average	228.2	51.2	80.1	101.58	115.3	14.2
% Laying	Front	67	30	39	32	42.0	9.0
	Mid	62	39	63	38	50.5	--
	Rear	71	32	38	45	46.5	21.0
	Average	66.7	33.7	46.7	38.3	46.4	15.0
% Laying days	Front	24.5	19.4	16.9	15.1	19.0	11.6
	Mid	29.7	16.9	20.0	19.4	21.5	--
	Rear	26.4	15.1	19.4	21.4	20.6	15.5
	Average	26.9	17.1	18.8	18.6	20.4	13.6

^a gross reproduction is total eggs laid by a hypothetical female that lived to the oldest age; net reproduction is eggs produced by the average female.

^b numbers in this column represent values for the two treatments in which three legs were amputated—the two middle legs plus the leg indicated for each row.