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Insects As Indicators of Environmental Stress

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INSECTS AS INDICATORS OF ENVIRONMENTAL STRESS

by

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Bachelor of Science
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DEDICATION

I would like to dedicate this body of work to the numerous people who guided and supported me throughout this process: my parents, Paul and Pam for always being there for me and loving me no matter what, my sister and brother, Erin and Anthony for reminding to have fun every once and a while, my Tai Chi teacher Sifu Wesley Adams for being such a good friend and a wise teacher and finally to my brothers and sisters at the Hall of the Snake and Crane. Thank you for the memories and friendships.

ABSTRACT

In the face of climate change-related events and anthropogenic disturbances, understanding the impacts of these events on species richness, abundance and distribution is important for us to mitigate biodiversity loss and better predict consequences for the environment and for human life. Insects are excellent models for understanding the consequences of environmental stress due to the sensitivity of their development and behavior. The overall focus of my dissertation research is the investigation of fluctuating asymmetry and oviposition behavior as indicators of environmental stress. My research includes a meta-analysis of published literature, and empirical research that was part of a collaborative initiative on understanding the ongoing ecological consequences of the 1986 Chernobyl disaster and the role of timber harvest activity on insect habitat selection. Finally, with the wider availability of Internet and GPS tracking technologies, my research investigated the relative efficacy of citizen science-based research methods on tracking a region-wide ecological event through collaboration with local research institutions. I will show that fluctuating asymmetry can serve as a reliable indicator of environmental stress and provide considerations for its application. I will also show how spatial distribution of citizen reports can relate to the ecological landscape and how changes in the landscape can impact insect behavior.

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CHAPTER 1

THE USE OF FLUCTUATING ASYMMETRY AS A MEASURE OF ENVIRONMENTALLY INDUCED DEVELOPMENTAL INSTABILITY[1]

Monitoring the impacts of environmental stressors on biological systems is of interest to biologists concerned with management and sustainability (Depledge and Galloway, 2005). Biomarkers are defined as functional measures of exposure to various stressors and can serve as an early warning system of declines in environmental quality and population health (Adams et al., 2001). They play a role in determining the presence of a stressor (Leung et al., 2003) and assessing the degree to which important taxonomic groups have been compromised (Schlenk, 1999).

Fluctuating asymmetry (FA) has become a popular method for measuring phenotypic response to environmental stress (Leamy and Klingenberg 2005). FA is defined as small, random deviations of bilateral traits from perfect symmetry due to subtle variations in the developmental environment (Palmer and Strobeck, 2003). Thus, significantly increased levels of FA in a population may indicate that individuals are having more difficulty maintaining precise development, resulting in negative effects on the population over time (Markow 1995). The attractiveness of FA as a potential biomarker stems from its non-lethal and broad application across biological systems, stressors and its expected association with life history traits and fitness (Lens et al. 2002; Lens and Eggermont,

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[1] Beasley, D.E., Bonisoli Alquati, A. and Mousseau, T.A. 2013. *Ecological Indicators*. 30: 218-226. Reprinted here with permission of publisher.

2008). An additional advantage of FA as a biomarker include the relative ease in taking measurements compared to other biomarkers that require more costly equipment (Leung et al., 2003). Given the difficulty in acquiring direct fitness measures from populations in the field, and the importance of physiological and developmental homeostases for fitness, researchers have proposed that FA serve as a surrogate for estimating how well a population is coping with changes in environmental conditions (Clark, 1995; Møller, 1997; Møller, 1999; Møller and Thornhill, 1997; Møller and Thornhill, 1998). However, results in the literature have been inconsistent across species, traits, and stressors, causing some to question FA's reliability as a biomarker (Clark, 1998; Floate and Coglin, 2010; Floate and Fox, 2000; Palmer, 1996). For example, Eeva et al. (2000) found increased levels of FA in the tarsus of great tits (*Parus major*) populations exposed to heavy metal contamination. However, a later study using the same species did not find an association between FA and heavy metal exposure (Dauwe et al. 2006). A similar contradiction arose when Valkama and Kozlov (2001) failed to detect an increase in FA levels in birch (*Betula pubescens*) leaves in response to air pollution, as shown by a previous study (Kozlov et al. 1996).

These inconsistent results may be due to a misuse of FA with respect to the study system. For instance, the setting of the study may influence the relationship between FA and the stressor. In field studies, organisms are exposed to many different environmental factors that may reduce the strength of the relationship between FA and the stressor in question. Similarly, different measures of FA may provide more or less detail regarding the morphology of the trait and its response to environmental stress. Studies that apply more sensitive methods, such as the use of geometric morphometrics for analyzing

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biological shape and size, may detect a larger signal compared to studies that use linear and/or meristic measures. Furthermore, while the general definition of a stressor encompasses *any* biotic or abiotic factor that interferes with an individual's energy allocation towards its reproduction and development, novel, anthropogenic stressors may have stronger effects on FA levels because they more actively interfere with developmental pathways (Parsons, 2005) and more directly limit the mass and energy available compared to naturally occurring stressors, against which adaptive responses are expected to have evolved (Graham et al., 2010; Hoffmann and Hercus, 2000). Additionally, if an environmental factor is not significantly interfering with the expression of fitness-related traits such as body size, the ability to complete development or developmental timing, it may not be a stressor at its current level. Thus, we would not expect FA to significantly vary in response.

Because of the increasing demand for simple biomarkers in conservation and monitoring studies (Forbes et al., 2006) and the potential use of FA as a general biomarker, we used a meta-analytical approach to assess if FA is a reliable biomarker of environmental stress. We also aimed to characterize the attributes of the different studies that explain their variation in the strength of the relationship between FA and environmental stress. We expect this analysis to identify the limitations of the application of FA as a reliable biomarker and provide directions concerning the study design and techniques that are more likely to detect a biological response of FA to a stressor.

Meta-analyses provide a systematic, detailed approach for identifying findings in common amongst studies, accounting for differences in study design and integrating results in a coherent manner (Arnqvist and Wooster, 1995; Gurevitch et al., 2001;

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Stewart, 2010). Additionally, by combining the results of multiple studies, they better identify the true magnitude of a small and weak relationship between two factors (Harrison, 2011). Thus, we collected a sample of studies that applied FA as a biomarker of environmental stress and determined the overall magnitude and direction of effect of stress on FA. We grouped studies by whether they conducted the experiment in the field or under laboratory conditions to determine if the degree of control for external factors outside of the experimental parameters affected the reliability of FA. Laboratory-based studies may be expected to detect larger effects due to the absence of confounding effects that could potentially dilute the strength of the relationship. Next, we grouped studies by the type of environmental stressor. We expected anthropogenic stressors such as heavy metal contamination and pesticides to have significantly larger and positive magnitudes of effect on FA compared to naturally occurring stressors such as changes in temperature. In naturally occurring stressors, organisms may be more developmentally resilient due to evolved adaptation compared to organisms exposed to novel stressors that interfere with an organism's ability to repair and maintain developmental pathways (Hoffmann and Hercus, 2000). We also grouped studies by the method in which they measured traits. We predicted that measurements that provide more detailed information about the morphology of the trait, such as geometric morphometric approaches to FA in shape and size, will be more sensitive to subtle changes in FA compared to meristic counts or linear measures. Finally, we categorized studies by whether or not they detected a negative fitness consequence via measures of fitness-related traits. We predicted that studies with evidence of a fitness consequence due to exposure to the environmental factor, verifying

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that the factor is indeed a stressor, would result in larger, more positive magnitudes of effect.

2. Material and Methods

2.1. Extracting data

We searched Web of Science (Web of Knowledge, Philadelphia, PA) and Google Scholar (Mountain View, CA) for articles using a minimum combination of two of the following keywords: 'fluctuating asymmetry' and 'environmental stress*', 'environmental disturbance*' and 'fluctuating asymmetry', 'environmental stress and 'insect'. We also searched the reference lists of the selected articles for additional studies that met our inclusion criteria. Our meta-analysis includes studies that: (1) used insect species, (2) addressed the question: 'does a certain environmental stressor affect FA?', and (3) applied FA as a biomarker of the impact of the stressor on the population. We limited the meta-analysis to insect studies because it is important to use a homogenous group of studies to better draw meaningful conclusions from the analysis (Hunter and Schmidt, 2004). We felt insects were an appropriate group to study because they have historically been used to assess the impacts of a variety of stressors on environmental quality. Insects have many characters such as rapid generation turnover, large samples and sensitivity to environmental changes that make them suitable bioindicators of environmental quality (McGeoch, 1998, 2007). We included the outcome for all traits measured in each study, as long as the trait met the statistical assumptions for FA analysis, which included absence of directional asymmetry, antisymmetry and trait-size dependence (Palmer and Strobeck, 2003). Thus, some studies contributed data for more

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than one effect size estimate. We excluded studies that examined the relationship between FA and mating success because the focus of those studies was not to apply FA as a biomarker. In total we found 53 studies, covering 42 species and yielding 179 relationships that met the search criteria (Table 1).

In order to include studies with different test statistics, we used correlation coefficients that we then used to estimate effect sizes (Cooper, 1998; Hunter and Schmidt, 2004; Rosenberg et al., 2000). Thus, student t-values from Levene's test, F-values from ANOVAs, chi-square values, Hedges' effect size values (g) from group mean comparisons and Spearman's correlation coefficients were all transformed into Pearson's correlation coefficients (r) (Defife, 2009; Rosenberg et al., 2000). If direct transformations were not available, we transformed the p-values into Z-scores and then transformed the values into our common summary statistic (r) (Leung and Forbes, 1996; Rosenberg et al., 2000).

2.2. Classification of studies

We first classified studies by whether they were conducted in the field or in laboratory conditions to test if studies under controlled conditions detected larger effects on FA. We also coded studies by whether or not the environmental stressor was anthropogenic to test if studies that assessed novel stressors that directly interfere with mechanisms of repair and maintenance detected larger effects on FA compared to studies that tested more naturally occurring stressors. To address if studies that applied a multidimensional measurement or a combination of trait measurements detected larger effects of the stressor on FA, we categorized studies by the kind of trait measured and the

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associated measurement method: meristic (counts), metric (linear) or composite (shape and size). Finally, because lack of a significant effect on FA in some studies may be due to the environmental stressor not being highly stressful for the study population, we categorized studies by whether or not they found a negative effect on fitness-related traits (i.e. fecundity, reduction in body size).

2.3. Meta-analysis

We calculated effect sizes for each relationship by transforming individual summary measures (r) with Fisher-z-transformation formula (Zr):

$$Zr = ½ \ln ((1+r)/(1-r))$$

and variance (v_z):

$$v_z = 1/(n-3)$$

where *n* is the sample size, using MetaWin 2.0 software (Rosenberg et al. 2000). We then used an unstructured random-effects model to calculate a mean effect size (ES) across all studies (Rosenberg et al., 2000). A random effects model allowed us to account for variation in effect sizes not explained by sampling error. This approach is recommended for meta-analyses involving data from ecological studies because the true effect size is expected to randomly vary due to ecological factors not tested (Gurevitch et al., 2001). We calculated 95% confidence intervals based on the parametric variance estimate of the mean effect size at the 2.5 and 97.5 percentile values. Additionally, we calculated bootstrap confidence intervals based on nonparametric variance around a sample of individual effect size estimates using a resampling test with 9999 iterations to provide a more conservative estimate of the mean effect size (Adams et al., 1997; Rosenberg et al.,

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2000). Mean effect sizes were considered to be significantly different from zero ($p <$ 0.05) if their confidence intervals did not include zero (Hunter and Schmidt, 2004). To test for different sources of variation amongst studies in the sign and magnitude of effect size of the relationship between the environmental stressor and FA, we classified studies based on explanatory variables (see *2.2. Classification of studies*) and examined between-group heterogeneity (Q_b) (Gurevitch et al., 2001). We assessed the total heterogeneity (Q_T) against a chi-square distribution to test how consistent the variance was across all effect sizes (Rosenberg et al., 2000). A significant Q_T indicates that variance amongst effect sizes is too different for meaningful comparison and suggests that other explanatory variables should be investigated (Cooper, 1998; Gurevitch et al., 2001).

We also visually inspected the data with a funnel plot of effect size estimates against sample size (Fig. 1). Additionally, to evaluate the robustness of our findings we applied a fail-safe calculation based on Rosenberg's method that provides a weighted fail-safe estimate (Rosenberg 2005). A large fail-safe number relative to the number of observed studies indicates that the observed results, even with publication bias, can be treated as a reliable estimate of the true effect (Rosenberg et al. 2000). Additionally, we can detect the presence of publication bias as a negative covariance between effect size and sample size (Begg 1994). Thus, we ran a regression analysis of effect size on sample size.

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3. Results

3.1. Mean effect size and tests for publication bias

We squared the value of our estimated mean effect size to judge the proportion of variance explained by environmental stress in the meta-analysis (R^2). Thus, effect sizes that are equal to or less than 0.10 are considered small (corresponding to 1% of the variance being explained), 0.30 are considered medium, and 0.50 indicates a large effect (Cohen, 1988). Typically, in meta-analyses of ecological and evolutionary biology studies, the proportion of variance explained by the main factor in question ranges between 5-10% (Møller and Jennions, 2002).

Overall, the mean effect size across all studies was 0.60 (95% CI = 0.50-0.70; Bootstrap CI = 0.52-0.68), indicating that environmental stressors accounted for 36% of the variance in FA. Variance across individual effect sizes was non-significant (Q_T = 116.6; d.f. = 178; X^2 = 1.00). Thus, with the overall large effect, we can expect FA to be highly affected by environmental stressors and therefore serve as a reliable biomarker of environmentally induced developmental instability.

Our weighted fail-safe estimate was 76, suggesting our findings are unlikely to be eliminated by publication bias. Furthermore, our regression analysis found effect size increased with sample size (F = 4.84, df = 1, 177, r^2 = 0.03, p = 0.03, slope (SE) = 1.89 x 10^{-1} (8.63 x 10^{-2})), which is contrary to the expectations of publication bias.

3.2. Classification of studies

We detected a significant difference between estimates that demonstrated a negative effect of the stressor on fitness-related traits compared to those that showed no effect of the stressor ($Q_b = 9.56$, d.f. = 1, p < 0.01). Studies where the stressor had a significant effect on fitness-related traits also tended to have the largest effect sizes (effect size (ES) = 0.70, d.f. = 123, 95% CI = 0.58-0.82, Bootstrap CI = 0.60-0.81, n = 34, k =123) when compared to studies that did not find such an effect (ES = 0.37, d.f. = 55, 95% CI = 0.19-0.55, Bootstrap CI = 0.29-0.45, n = 18, k = 56; Fig. 2). Thus, our estimation of the sensitivity of FA as a biomarker of exposure to an environmental stress is likely to be conservative, as studies that did not find an effect of the stressor on FA might have dealt with a factor that was not affecting the study species at all.

When we grouped studies by whether they were conducted under field or laboratory conditions, we detected significant variation across ($Q_b = 4.91$, d.f. = 1, p = 0.01), with laboratory-based studies having larger effect sizes (ES = 0.66, d.f. = 131, 95% CI = 0.55-0.78, Bootstrap CI = 0.56-0.77, n = 39, k = 132) compared to field studies (ES = 0.41, d.f. = 46, 95% CI = 0.22-0.61, Bootstrap CI = 0.32-0.50, n = 14, k =47) suggesting that the conditions under which the study was conducted explain some of the variation amongst studies (Fig. 3). Next, we tested if the type of environmental stressor accounted for the variation in FA response and did not detect any significant variation according to whether the stressor was naturally occurring or anthropogenic ($Q_b = 0.60$, d.f. = 1, p = 0.34; Table 2). Finally, we did not find a significant effect when we classified studies by the kind of trait that they measured ($Q_b = 0.72$, d.f. = 2, p = 0.58; Table 3).

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4. Discussion

Our study used a meta-analytical approach to assess the reliability of FA as a biomarker of environmentally induced developmental instability, and to identify features of different studies that might explain variation in FA response to changes in environmental quality. Overall, we found that FA can serve as a sensitive biomarker, with environmental stress having a very large effect on FA response in our sample of studies. Particularly, studies that verified a negative effect of the stressor on fitness-related traits detected significantly larger effects. Studies conducted under the controlled conditions of a lab detected comparatively larger effects of the environmental stressor on FA than studies of wild populations. Other study factors such as method of measuring traits and the nature of the stressor did not significantly explain variation amongst studies, suggesting recent improvement in analytical tools and sampling considerations have standardized and enhanced the rigor of FA analysis across insect studies (Palmer and Strobeck, 2003).

As a biomarker, FA is particularly advantageous due to the ease in identifying optimal levels (i.e. perfect symmetry) compared to physiological biomarkers. Determining the optimum level of physiological biomarkers is complicated for two reasons: one, the up regulation of these biomarkers usually occurs at the expense of their availability for other functions. For example, antioxidants in birds play an important role in both immune function and the coloration of secondary sexual characters and has recently been used as an indicator of exposure to radiological contaminants (Bonisoli Alquati et al., 2010; Peters, 2007). Studies have demonstrated how reduced availability of carotenoids due to increased immune activity resulted in paler color expression in

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secondary sexual characters (Aguiliera and Amat, 2007; Alonso-Alvarez et al., 2004). Thus, with multiple biological functions influencing the levels of antioxidants, it may be difficult to determine when levels indicate a positive or negative response to a stressor. Two, while short-term response of some physiological biomarkers to a stressor is adaptive, it may prove to be detrimental in the long term if the stressor persists (Dhabhar, 1999). For instance, inflammatory immune responses are known to cause tissue damage over the long term and prolonged exposure to glucocorticoids causes significant damage to the biological system (Maccari et al., 2003).

Additionally, FA is a less invasive measure of quality compared to more invasive techniques that require removing tissue or killing the animal. For example, one of the better-known molecular biomarkers, cytochrome P450, has proven to be a very reliable indicator of exposure to organochlorine contaminants (Sarkar et al., 2006). However, the use of this biomarker has traditionally required the harvesting of liver tissue from dead animals, presenting legal and ethical concerns for conservation studies (Miller, 2003). In the case of heat shock proteins, technical issues such as the availability of antibodies for specific species limits the use of these stress proteins for environmental assessment (Sørensen, 2010).

The fitness consequences associated with elevated levels of FA in addition to its sensitivity to environmental stress is important for conservation studies (Depledge and Fossi, 1994; Møller, 1997). Schmeller et al. (2011) found that variance in population-wide FA in the wing veins of the Mountain Apollo Butterfly (*Parnassius apollo)*, an endangered butterfly species, was comparable to FA variance of pre-threatened populations 13 years after management practices went into effect, supporting the use of

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FA as an assessment tool, although the potential effects on FA related to inbreeding in small populations must also be assessed. Additionally, Monna et al. (2011) used FA to assess developmental quality in wild brown trout (*Salmo trutta fario*) exposed to heavy metal pollution in a protected area that historically was a site for heavy mining activity. The high levels of FA in populations in relation to high metal contamination emphasized the importance of considering an area's contamination history when implementing long-term management plans.

The association of the increased FA with negative effects on fitness-related traits emphasizes the importance of considering the evolutionary potential of populations and may offer insight into why results in the application of FA have been inconsistent in the literature. There is increasing recognition for the incorporation of evolutionary ecology in conservation management due to the need for understanding how global environmental changes and management practices influence a species' adaptive response (Davis et al, 2005; Stockwell et al., 2003). Hoffmann and Willi (2008) presented evidence demonstrating changes in candidate loci responsible for adaptive responses, such as the alcohol dehydrogenase gene (*Adh*) in the fruit fly (*Drosophila melanogaster*) and the glycerate dehydrogenase locus (*Gly*) in Piñon Pine (*Pinus edulis*), with various environmental stressors and climate change. Thus, interpretation of FA should be considered within the limits of the population's adaptive potential and current understanding of a population's life history. For example, Hogg et al. (2001) looked at FA as a biomarker of water temperature stress while considering the underlying population genetics of the stonefly (*Nemoura trispinosa*) and detected a negative correlation between levels of FA and heterozygosity. Lens et al. (2000) found similar

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results when they assessed FA as a biomarker of habitat disturbance in populations of Taita thrush (*Turdus helleri*) with reduced heterozygosity. Levels of FA were more pronounced in highly disturbed areas but weak under less disturbed conditions.

We predicted that lab studies would detect larger effects of the environmental stressor on FA as a result of fewer factors interfering with the strength of the relationship. A previous meta-analysis on FA and environmental stress suggested that the lack of biological validation of the stressor could dilute the strength of the association (Leung & Forbes, 1996). This is more difficult to determine in wild populations that are exposed not only to the stressor in question but to additional factors that have unknown effects on the study organism. Additionally, our finding that lab-based studies detected larger magnitudes of effect of the environmental factor on FA, further emphasizes the importance of considering the population's adaptive potential in relation to its response to stress. For instance, stronger effects of stressors on FA may also be attributed to reduced genetic diversity for stress tolerance in lab populations while wild populations with more genetic diversity have more adaptive potential and comparatively lower FA as a result. Frankham (2005) found that loss of genetic variation in *Drosophila melanogaster* raised in controlled conditions reduced stress resistance and significantly suppressed the evolutionary adaptive potential in stressful environments when populations were re-introduced into the field. Thus, lab studies such as artificial selection experiments may provide insight into understanding FA response to environmental stressors in relation to adaptation and trade-offs with stress resistance and fitness-related traits in vulnerable populations (Bijlsma and Loeschcke, 2005).

Our study showed larger strengths of association between environmental stress and FA compared to previously published meta-analyses, which included multiple taxa (Leung & Forbes 1996; Hogg et al., 2001). One possible reason may be that primary studies on mammals or fish, where larger sample sizes are difficult to obtain, are more likely to be hampered by measurement error, other asymmetry types and trait choice, compared to studies on insects (Allenbach, 2010). Another consideration is that the life history of many insects leaves them comparatively more susceptible to environmental changes, making the taxa a more suitable model for using FA to evaluate environmental quality. Additional studies on how FA responds within taxa will help better understand the general use and interpretation of FA across taxa.

Finally, our study did not find a significant difference between naturally occurring and anthropogenic stressors in causing FA. This is in contrast to a previous study that detected a significant association between toxic stressors and FA (Hogg et al., 2001). Our results suggest that the duration of exposure may have greater influence on FA. However, formulating predictions are difficult given that prolonged exposure to a stressor can cause either developmental selection for symmetry that would reduce levels of FA in the population or increase the population's mutational load that would impair development and thus increase FA. Studies that look at the change in level of FA as the selection pressure increases could help determine the role of prolonged exposure when using FA to assess environmental stress (Polak et al., 2002).

In conclusion, the use of FA as a biomarker may be most informative when a negative effect of the stressor exists on fitness-related traits, verifying that the environmental factor in question is indeed a stressor. To further our understanding on the use and

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limitations of FA as a biomarker, future studies may wish to clarify how FA's response to environmental stress varies over the course of exposure within a population. Such a study could potentially expand into multi-generational responses of FA to a stressor, and insect models would be ideal (e.g. Beasley et al., 2012). This is particularly important for contaminants such as oil spills, radiological and metal pollutants that are expected to persist in the environment for long periods of time. Studies looking at FA in relation to adaptive responses of a population will provide additional insight to how FA co-varies with stress in the context of evolutionary constraints and plasticity.

Table 1: Studies on the effect of environmental stress on fluctuating asymmetry included in the meta-analysis: insect species, stressor, stressor type, stressor-fitness relationship, trait measurement, number of traits, experimental environment, sample size and reference.

Species	Stressor	Stressor Type	Stressor-Fitness Relationship	Trait Measurement	Number of Traits	Experimental Environment	Sample Size	Reference
Apis mellifera	Pesticides	Anthropogenic	No Effect	Metric	3	Field	180	Abaga et al., 2011
Drosophila melanogaster	Endosulfane exposure	Anthropogenic	Negative Effect	Metric Meristic	2	Lab	220, 221	Antipin and Imasheva, 2001
Cyrtodiopsis dalmanni	Temperature	Natural	Negative Effect	Composite	1	Lab	120	Bjorksten et al., 2001
Hydropsyche morosa	Water pollution	Anthropogenic	No Effect	Metric	5	Field	40	Bonada and Williams, 2002
Hydropsyche exocellata	Water pollution	Anthropogenic	No Effect	Metric	3	Field	165	Bonada et al., 2005
Coenagrion puella	Parasitism	Natural	No Effect	Metric Meristic	2	Field	148	Bonn et al., 1996
Lygus hesperus	Nutrition	Natural	Negative Effect	Composite	1	Lab	3293	Brent, 2010

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Coenagrion puella	Atrazine Carbaryl Endosulfan	Anthropogenic	Negative Effect	Metric	1	Lab	300	Campero et al., 2007
Cephus cinctus Norton	Wheat host variation	Natural	Negative Effect	Metric	5	Lab	171	Carcamo et al, 2008
Copera annulata	Temperature Avermectin B Imidacloprid	Anthropogenic	Negative Effect	Metric	5	Lab	35-39	Chang et al., 2007
Ceriagrion sp.	Insecticide	Anthropogenic	Negative Effect	Metric	3	Lab	22	Chang et al, 2007
Copera annulata	Avermectin B Imidacloprid	Anthropogenic	No Effect	Metric	8	Lab	44-58	Chang et al., 2009
Musca vetustissima	Avermectin B	Anthropogenic	Negative Effect	Metric	1	Field	420	Clarke and Ridsdill-Smith, 1990
Chrysopa perla	Water pollution	Anthropogenic	Negative Effect	Meristic	1	Field	161	Clarke, 1993
Haematobia irritans	Diflubenzuron	Anthropogenic	Negative Effect	Metric	2	Lab	109	Da Silva et al., 2004
Hexagenia rigida	PCB	Anthropogenic	No Effect	Metric	3	Field	30	Dorbin and Corkum, 1999

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Scathophaga stercoraria	Ivermectin	Anthropogenic	No Effect	Metric	7, 12	Lab	30, 60	Floate and Coghlin, 2010
Musca domestica L.	Ivermectin	Anthropogenic	Negative Effect	Metric	1	Lab	178	Floate and Fox, 2000
Pararge aegeria L.	Larval density	Natural	Negative Effect	Composite	1	Lab	280	Gibbs and Breuker, 2005
Brevicoryne brassicae	Lead accumulation in radish and cabbage plant Copper accumulation in radish and cabbage plant	Anthropogenic	Negative Effect Negative Effect	Metric	4	Lab	120	Gorur, 2006
Brevicoryne brassicae	Zinc Accumulation in Radish and Cabbage Plant Cadmium Accumulation in Radish and Cabbage Plant	Anthropogenic	Negative Effect	Metric	4	Lab	120	Gorur, 2009
Drosophila melanogaster	Lead and benzene exposure	Anthropogenic		Meristic	1	Lab	960	Graham et al., 1993
Chironomus riparius	Metal-contaminated river	Anthropogenic	No Effect	Meristic	1 (per site)	Field	620 (field)	Groenendijk et al., 1998

								Lab	334 (lab)	
Xanthocnemis zealandica	Carbaryl exposure	Anthropogenic	No Effect	Composite	1	Lab	72	Hardensen et al., 1999
Xanthocnemis zealandica	Carbaryl exposure	Anthropogenic	No Effect	Metric	3	Lab	141	Hardensen, 2000
Xanthocnemis zealandica	Short term pesticide exposure	Anthropogenic	Negative Effect	Composite Metric Meristic	3	Lab	40	Hardersen and Frampton, 1999
Xanthocnemis zealandica	Carbaryl exposure	Anthropogenic	No Effect	Meristic Composite Metric	3	Lab	57	Hardersen and Wratten, 1998
Helicoverpa punctigera	Pyrethroid esfenvelerate exposure Cold temperature exposure	Anthropogenic Natural	Negative Effect	Composite	4	Lab	73	Hoffmann et al., 2002
Nemuora trispinosa	Temperature	Natural	Negative Effect	Meristic Composite	2	Field	172	Hogg et al., 2001
Scathophaga stercoraria	Temperature Density	Natural	Negative Effect	Composite	4	Lab	64	Hosken et al., 2000

Species	Stressor		Effect	Type		Setting	Sample	Reference
Drosophila pachea	Cactus host variation	Natural	Negative Effect	Metric	2	Lab	84, 86	Hurtado et al., 1997
Drosophila melanogaster	Nutrition	Natural	Negative Effect	Metric	3	Lab	539	Imasheva et al., 1999
Drosophila melanogaster *Drosophila buzzatti*	Temperature	Natural	Negative Effect	Metric Meristic	3 (per species)	Lab	300	Imasheva et al., 1997
Drosophila subobscura	Lead exposure	Anthropogenic	No Effect	Metric	1	Lab	32	Kurbalija et al., 2010
Pterostichus melanarius	Fungicides exposure Insecticide exposure	Anthropogenic	Negative Effect	Metric Meristic	3	Field	587	Labrie et al., 2003
Culex pipiens	Temperature	Natural	Negative Effect	Metric	3	Lab	109,341,376	Mpho, 2002
Culex quinquefasciatus	Density	Natural	Negative Effect	Metric	4	Lab	35, 144	Mpho et al., 2000
Culex quinquefasciatus	Temperature	Anthropogenic	Negative Effect	Composite	6	Lab	11, 12, 19, 22, 24	Mpho et al., 2001
Argia tinctipennis Selys	Riparian vegetation removal	Natural	Negative Effect	Metric	10	Field	70	Pinto et al., 2012

				Meristic				
Drosophila melanogaster	Arsenic	Anthropogenic	No Effect	Merisitic	2	Lab	1229	Polak et al., 2001
Drosophila melanogaster	Temperature Lead exposure	Natural Anthropogenic	Negative Effect No Effect	Meristic	4	Lab	686	Polak et al., 2004
Formica pratensis Retz.	Metal-contaminated sites near smelting plant	Anthropogenic	No Effect	Metric	4	Field	350	Rabitsch, 1997
Chironomus riparius	Seasonal and temporal variation urban and industrial wastewater	Anthropogenic	No Effect	Metric Meristic Composite	4	Field	44	Servia et al., 2004
Eulaema nigrita Lepeletier *Euglossa pleosticta Dressler*	Seasonal Variation Anthropic influence	Natural Anthropogenic	No Effect Negative Effect	Metric	4	Field	60	Siliva et al., 2009
Drosophila buzzatti *Drosophila koepferae*	Catcus host variation	Natural	Negative Effect	Composite	4	Lab	53, 55	Soto et al., 2008

Drosophila antonietae	Plant Host	Natural	Negative Effect	Composite	2	Lab	325	Soto et al., 2010
Pararge aegeria L.	Nutrition	Natural	Negative Effect	Composite	1	Lab	581	Talloen et al., 2004
Pleioplectron simplex	Parasitism	Natural	No Effect	Metric	3	Lab	65	Thomas et al., 1998
Pleioplectron simplex	Parasitism	Natural	No Effect	Metric	1	Lab	32	Thomas et al., 1998
Drosophila melanogaster	Temperature	Natural	Negative Effect	Metric	1	Lab	35	Trotta et al., 2005
Dacus dorsalis	Methyl eugenol exposure	Anthropogenic	Negative Effect	Metric	1	Field	48	Tsubaki, 1998
Drosophila ananassae	Temperature	Natural	Negative Effect	Metric Meristic	5	Lab	600	Vishalakshi and Singh, 2008
Parellipsidion pachycercum Celatoblatta quicquemaculata Sigaus obelisci Paprides dugdali	Parasitism	Natural	No Effect	Metric	1 (per species)	Lab	34	Ward et al., 1998

Phaulacridium marginale *Concephalus bilineatus*								
Scathophaga stercoraria	Avermectin B	Anthropogenic	No Effect	Metric	1	Field	113	Webb et al., 2007

Table 2: Mean effect sizes (ES) , confidence intervals (CI), number of studies (n), number of relationships presented in the study (k), heterogeneity among studies (Q_T), degrees of freedom (df = k-1), chi-square distribution (x^2) grouped by environmental stressor type: anthropogenic and natural. Note that some studies contributed data for more than one category so n-values do not sum up to total number of studies in meta-analysis.

Stressor Type	Mean ES	95% CI	Bootstrap CI	n	k	Q_T	df	x^2
All studies	0.60	0.50 , 0.70	0.52 , 0.68	54	179	121.2	178	1.00
Anthropogenic	0.56	0.43,0.70	0.48,0.66	30	102			
Natural	0.64	0.49,0.79	0.50,0.79	22	77			

Table 3: Mean effect sizes (ES) , confidence intervals (CI), number of studies (n), number of relationships presented in the studies (k), heterogeneity among studies (Q_T), degrees of freedom (df = k-1), chi-square distribution (x^2) grouped by measures of traits: metric, meristic and composite. Note that some studies contributed data for more than one category so n-values do not sum up to total number of studies in meta-analysis.

Trait Measure	Mean ES	95% CI	Bootstrap CI	n	k	Q_T	df	x^2
All studies	0.60	0.50 , 0.70	0.52 , 0.68	54	179	116.6	178	1.00
Metric	0.57	0.44,0.69	0.47,0.67	19	111			
Meristic	0.68	0.43,0.93	0.44,0.94	5	30			
Composite	0.63	0.40,0.85	0.51,0.75	6	38			
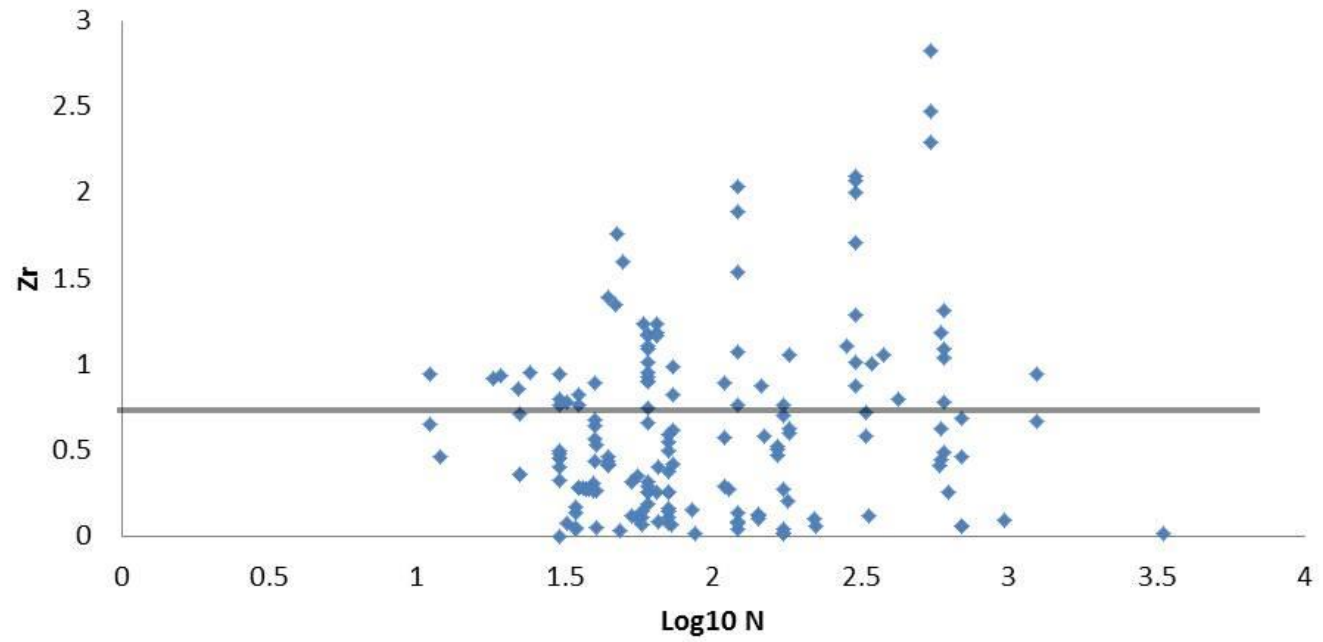
Figure 1.1. Funnel plot of Fisher-z-transformed effect sizes in relation to sample size
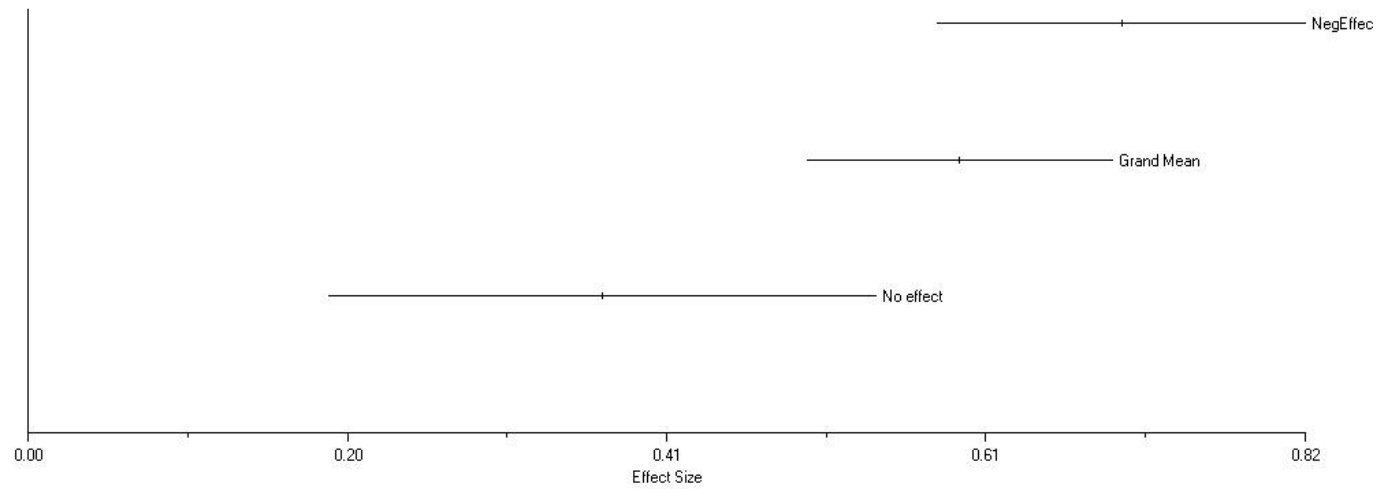
Figure 1.2. Forest plot of mean effect sizes categorized by the effect of stressor on fitness-related traits (negative effect vs. no effect) in order of increasing effect size. Effect sizes are z-transformed Pearson correlation coefficient estimates and shown with 95% confidence intervals. $Q_T = 120$, df $= 178$, $x^2 = 1.00$
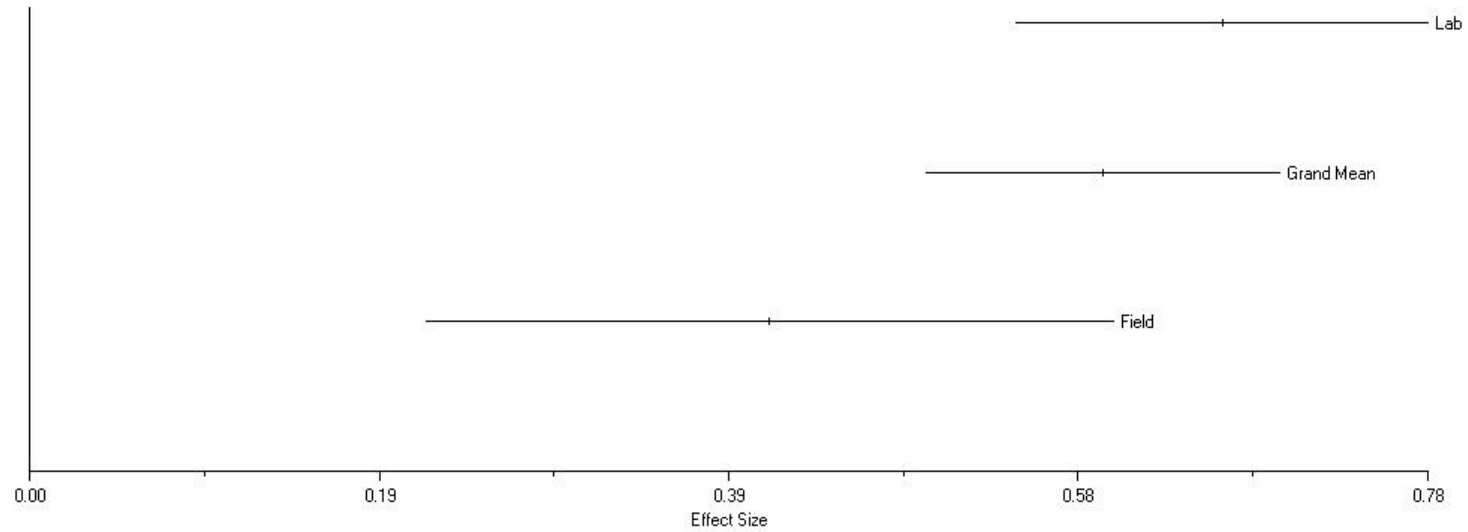
Figure 1.3. Forest plot of mean effect sizes categorized by experimental environment (field vs. lab) in order of increasing effect size. Effect sizes are z-transformed Pearson correlation coefficient estimates and shown with 95% confidence intervals. $Q_T = 118.7$, df = 178, $x^2 = 1.00$

CHAPTER 2

EFFECTS OF PARENTAL RADIATION EXPOSURE ON DEVELOPMENTAL INSTABILITY IN GRASSHOPPERS[2]

Genomic instability in the form of chromosomal irregularities or genetic mutations can occur because of environmental stressors such as viruses (Wu et al., 2010), plant toxins (Eizirik et al., 1995), mutagenic chemicals (Mishra et al., 2009) and radiation exposure (Dubrova, 2003). This instability occurs in mitotically dividing cells and interferes with the function of tissues and organs (Wong et al., 2003). Transgenerational genomic instability results from genomic instability transferred from the parental germline to the somatic cells of offspring, leading to the destabilization of developmental pathways (Natarajan, 2006). Exposure to external stressors and trans-generational genomic instability, which increases sensitivity of the progeny genome to mutagens (Hoyes et al., 2001), can have developmental and fitness consequences for individuals inhabiting stressful environments.

Exposure to radiation was one of the first external factors observed to induce transgenerational genomic instability in mammalian models (Sinclair, 1964; Nomura, 1982; Lyon et al., 1988; Luke et al., 1997; Morgan, 1999). Additionally,

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[2] Beasley, D.E., Bonisoli-Alquati, A., Welch, S.M., Møller, A.P. and Mousseau, T.A. 2012. *Journal of Evolutionary Biology.* 25(6): 1149-1162. Reprinted here with permission from publisher.

transgenerational effects from radiation exposure have also been demonstrated in insects, particularly in the form of inherited sterilization (North and Holt, 1968, 1969; Bloem et al., 1999; Carpenter et al., 2009). Ionizing radiation generates reactive oxygen species (ROS) either by the oxidation of water or secondarily by producing reactive intermediates (Rugo et al., 2002). Accumulation of ROS can result in oxidative stress, defined as the imbalance between ROS generation and the antioxidant defenses that neutralize them (Monaghan et al., 2009), which can cause an increase in mutation rates (Riley, 1994). More recently, radioactivity has been linked to inhibition of standard epigenetic processes such as methylation (Koturbash et al., 2006), histone modification (Rogakou et al., 1998) and RNA-associated silencing (Jaenisch et al., 2003), which may result in genomic instability (Kovalchuk et al., 2008). These mutagenic and epigenetic effects of ionizing radiation and its lingering effects across generations could lead to amplification of natural genetic variation and provide a source of heritable phenotypic variation (Vasileva et al., 1997; Johannes et al., 2009). Thus, radiation effects may serve as a model for understanding the mechanism producing phenotypic evolution of a population in a stressed environment (Badyaev, 2005).

The effects of chronic radiation exposure on growth, development and reproduction have historically been the subject of numerous studies (Marshall, 1962; Brown, 1964; Woodwell, 1967; Woodwell & Rebuck, 1967; Geras'kin et al., 2007). These studies have shown adverse effects on the fitness, longevity and health of living organisms (Zaire et al., 1997; Chen et al., 2001; Nicastro et al., 2002; Møller et al., 2005; Møller & Mousseau 2007; Ryabokon & Goncharova 2006; Veremeyeva et al., 2010). Human populations exposed to high radionuclide contamination have shown increased

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rates of germline instability (Dubrova et al., 1998), transmissible, clastogenic factors from exposed cells that damage unexposed cells (i.e. bystander effects) (Marozik et al., 2007), and elevated chromosome alterations (Neronova et al., 2003). Recently, Aghajanyan & Suskov (2009, 2010) demonstrated increased levels of abnormal cells and chromosomes in children born after the Chernobyl accident to irradiated fathers (i.e. liquidators) and non-irradiated mothers. These aberrations were analogous to the cytogenetic changes observed in children living in areas contaminated with radionuclides (Aghajanyan & Suskov 2009, 2010). These results are consistent with previous findings of children of Chernobyl liquidators conceived after the accident showing high genomic DNA mutation levels that decreased with elapsed time between exposure and conception (Weinberg et al., 2001). These studies imply transgenerational consequences of low-dose radiation exposure although the degree of the effect remains unclear (Slebos et al., 2004; Furitsu et al., 2005).

Conditions in areas such as Chernobyl may serve as a natural laboratory for studying the effects of transgenerational genomic instability on fitness in populations under chronic radiation exposure. On April 26, 1986, Reactor Four of the Chernobyl nuclear plant exploded during a test of the reactor's ability to generate power during an unplanned shut down. The resulting explosion released between 9.53×10^3 petabecquerel (PBq) and 1.25×10^4 PBq of radionucleotides into the atmosphere (Konstantinov & Gonzalez, 1989; European Union, 1998; Chernobyl Forum, 2005). Radioactive fallout dispersed across large portions of the Soviet Union and Europe, particularly Ukraine, Belarus, and Russia (Shestopalov et al., 2003). Most of the radionuclides dissipated

within days, but Cesium-137, Strontium-90 and Plutonium-239, among others, still persist in the environment to this day (Yablokov et al., 2009).

Previous ecological studies in Chernobyl have demonstrated fitness and developmental consequences due to radiation exposure in a variety of vertebrate and invertebrate species. Barn swallows (*Hirundo rustica)* in the Chernobyl area have shown increased mutation rates (Ellegren et al., 1997), increased frequency of partial albinism (Ellegren et al., 1997; Møller & Mousseau 2001), sperm abnormalities (Møller et al., 2005, 2008), increased DNA damage and oxidative stress (Bonisoli-Alquati et al. 2010 a, b), reduced levels of circulating and stored antioxidants (Møller et al., 2005) and elevated levels of fluctuating asymmetry in tail length (Møller 1993) in relation to radiation exposure. A study on stag beetles (*Lucanus cervus)* in Chernobyl found that males in highly contaminated sites had significantly elevated levels of fluctuating asymmetry in secondary sexual characters compared to males from control sites, which affected mating status as a larger portion of asymmetric than symmetric males were unmated (Møller 2002). Additionally, studies of human populations in areas such as the Semipalatinsk Nuclear Test Site and the Techa River in Southern Urals, Russia, where radioactive waste is dumped, showed similar increases in mutation rates in the germline as a result of long-term radiation exposure (Dubrova et al., 2002; Dubrova, 2006). Consistently, a decline in populations of both vertebrate and invertebrate species has been shown in radioactively contaminated areas in Chernobyl (Møller & Mousseau 2007, 2009, 2011). This is particularly apparent in invertebrate species that live or lay eggs in the topsoil where most of the radiation is currently located, suggesting a negative effect of direct exposure to radiation (Møller & Mousseau 2009).

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The aim of this study was to assess the effect of parental radiation exposure on offspring development under common garden conditions. We expected to see a comparable reduction in fitness across differing radiation levels reflected in delayed development and reduced body size at reproductive maturity in offspring derived from high radiation sites. In addition, it could be expected that disruption of development would be buffered by longer developmental times (Shykoff & Møller 1999). Therefore the effect of parental exposure to radioactive contamination is predicted to depend on the developmental trajectory, as indexed by time to sexual maturity. We used variation in wing shape and size and fluctuating asymmetry (FA) as proxies for the magnitude of developmental instability among offspring (Palmer & Strobeck, 1986, 2003; Klingenberg & McIntyre, 1998). FA reflects the inability of the individual to undergo identical development of bilateral traits under a wide range of environmental and genetic stressors (Møller, 1993; Swaddle et al., 1994; Graham et al., 2010). Additionally, a negative correlation between degree of FA and fitness components has been observed in many species (Møller 1992, 2002; Nosil & Reimchen, 2001; Mateos et al., 2008; St. Amour et al., 2010), suggesting that FA may be a reliable measure of phenotypic quality (Leary & Allendorf 1989; Parsons 1990; Møller & Thornhill, 1998), though its ability to reliably reflect fitness has been criticized in the literature (Palmer, 1999- but see Thornhill et al., 1999l Leung et al., 2003; Van Dongen, 2006; Floate & Coghlin, 2010). We predict that the offspring of parents from relatively high radiation sites would show high levels of asymmetry in bilateral traits compared to offspring from low radiation sites, as long as radiation did not cause increased rates of selective mortality of asymmetric individuals, measured as the

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number of nymphs that failed to reach adulthood in the irradiated and non-irradiated groups (Floate and Fox, 2000).

2.1. Materials and methods

2.1 a. Study species and field procedures

Studies on the effects of irradiation on insect reproduction and development have shown Orthopterans to be particularly radiosensitive (Willard & Cherry, 1975; Bakri et al., 2005). The brown form of the lesser marsh grasshopper (*Chorthippus albomarginatus* (Acrididae: Orthoptera)) is a medium sized grasshopper that has undergone a northward range expansion within the last 10-15 years (Bellmann, 1985; Vedenina & von Helversen, 2003). It is found in agricultural areas in dry and wet soil throughout Europe from the southern half of Scandinavia and Finland to Spain and Italy (Bellmann, 1985) along coastal sand dunes, rivers and roadside verges. The species has a univoltine life cycle with an obligate egg diapause stage for overwintering. Nymphs typically emerge between May and June and develop through 4-5 nymphal instars. Adults occur from July to October.

We field collected *C. albomarginatus* from six different locations in the Chernobyl area during 20 – 26 September 2009, across a range of radioactive contamination levels by a factor of 1670 (Fig. 1; Table 1). The six locations were Chystohalivka (0.03 μSv/h), Rozizhdzhe (0.1 μSv/h), Vesniane (5.4 μSv/h), Krasnytsia (10.72 μSv/h), and two sites within the Red Forest (35.31 μSv/h and 50.06 μSv/h, respectively). Background radiation levels were measured using a hand held dosimeter (Inspector, SE International, Summertown, TN, USA). Although we did not measure the dose received by individuals,

we expect it to be highly correlated with background radiation levels measured at the collection sites due to the short dispersal distance of our model species compared to the scale at which radioactive contamination levels vary (Fig.1). Individuals were collected from each collection site using a standard insect sweep net. Females were placed individually in Styrofoam cages (height ca. 12 cm, diameter ca. 8 cm) by collection site and provided with sterilized sand for ovipositing and fresh lettuce. Plastic cups (height ca. 10 cm, diameter ca. 8 cm) were used to enclose the top of the cages. Following removal from breeding cages, the number of egg pods per female was counted and they were placed in vials containing moistened vermiculite to allow for the completion of pre-diapause embryonic development.

Upon returning to the lab, we placed the egg pods in a 4°C refrigerator to break diapause. Egg pods were removed from the refrigerator following a three-month cold treatment and stored at room temperature (24°C) to induce post diapause embryonic development. Egg pods were monitored twice daily and newly emerged nymphs were transferred to 9 cm x 9 cm x 8 cm plastic cages. Cage density averaged 9 nymphs per cage (range: 2-18 nymphs) over the duration of nymphal development. Families with large numbers of nymphs (> 10) were divided into two cages. Cages were changed and fresh food provided twice weekly. Nymphs were fed organic lettuce, carrots and wheat germ and provided with a strip of unbleached paper towel for cover. We used carbon dioxide (CO_2) to transfer nymphs to clean cages with minimum mortality. The few individuals that died due to accidental mishandling during the rearing process were excluded from the analysis. Nymph cages were transferred to an incubator set at a constant temperature and photoperiod (28°C, 15:9 hours LD) to complete development.

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2.1 b. Life history estimates

We measured time to sexual maturity (time to maturity hereafter) as the number of days from nymphal hatch to final ecdysis. We also recorded body mass at sexual maturity (body mass hereafter) and the pronotum length (accuracy 0.1 mm) as measures of adult body size. Nymphs were monitored daily for final eclosure (maturity). Adults were removed from nymphal feeding cages and weighed with a Sartorius Research electronic balance (Model Number: R160P) (0.001 g) within ca. 12 hours after emergence. We then placed adults in individual cages with organic lettuce (~3.0 g) and a carrot slice (~9.0 g). We transferred adults to clean cages and provided fresh food twice weekly to maintain a clean environment. Following death, adults were removed from cages and stored in 95% ethanol for subsequent measurements.

2.1 c. Wing measurements

The forewings and hind wings were removed from the thorax and wet mounted on glass microscope slides with distilled water and covered with a cover slip. Slides were allowed to dry overnight at room temperature (24°C) and then cover slips were sealed to the slide with clear nail polish.

Slides were scanned face down with an Epson V300 Photo Color Scanner set at a resolution of 2400 dpi. Because measurement precision is important for the analysis of fluctuating asymmetry (Palmer & Strobeck, 1986, 2003), on a subsample of 20 individuals we scanned wings three times to assess scanning error using a Procrustes ANOVA with wings as the main effect, number of scans as the error effect and identity of specimens as the individual effect (Klingenberg, 2011). All landmarks were located at

wing-vein intersections and termination points (Fig. 2). Most importantly, landmarks were anatomically homologous amongst all individuals, thus fulfilling the criterion for Type I landmarks (Bookstein, 1991). Landmarks were digitized on the left and right hind wing using TpsDig2.16 software (Rohlf, 2005). All wings within the sample were measured three times to allow assessment of digitizing error with a Procrustes ANOVA with wings as the side effect, digitizing as the error effect and ID as the individual effect (Klingenberg, 2011). Individuals were digitized in random order, blind to information on parental exposure to radiation. The Procrustes ANOVA found the between sides variation to far exceed the measurement error due to the digitizing $(F_{72} = 12.09, P < 0.0001, r^2 = 0.97)$ and scanning technique $(F_{72} = 17.20, P < 0.0001, r^2 = 0.99)$ (Klingenberg, 2011). Additionally, we ran an analysis of error due to our mounting technique by wet mounting 54 wings on glass microscope slides and covering them with a cover slip before scanning. We repeated the process three times and then measured the length of the radial vein (pixels) twice for each wing using TpsDig2.16 software (Rohlf, 2005). We used a one-way ANOVA with vein length as our response variable and individual as our independent variable to assess the repeatability of our measurements. Measurement error due to mounting was very low for both the left wing $(F_{26} = 699.23, P < 0.0001, r^2 = 0.99)$ and right wing $(F_{26} = 245.86, P < 0.0001, r^2 = 0.99)$ with repeatability in excess of 99.43%. In the final analyses, we used mean landmark coordinates calculated by averaging the three measurements per landmark for each individual.

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2.1 c. Statistical analysis

Only the individuals for whom both wings could be measured were included in the final analysis. Our analysis included 454 individuals (223 males and 231 females). We extracted shape and size information from the raw data with the software MorphoJ (Klingenberg, 2011) using a full Procrustes fit that controlled for wing orientation, position and size (Klingenberg & McIntyre 1998; Klingenberg, 2002). We ran a Procrustes ANOVA to obtain mean individual values of centroid size as our measure of size variation, and two measures of shape variation: Procrustes distance and Mahalanobis distance. Procrustes distance is the absolute measure of shape variation that treats all aspects of shape variation equally (Klingenberg, 2005). Mahalanobis distance is a measure of shape variation relative to the variation in the total sample, with emphasis on degree of invariance (Klingenberg, 2005). Thus, this measure can be interpreted as the degree to which shape variation is unusual within the population of individuals being analyzed (Dryden, 1998; Klingenberg, 2005; Breuker et al., 2006). Centroid size is the square root of the sum of squared distances of a set of landmarks or the center of mass of the landmarks. It is the size measure used to scale a configuration of landmarks so they can be fitted in a frame of space, ultimately removing translation, size differences and rotation from shape analysis (Rohlf, 1999; Klingenberg, 2005). To further investigate shape variation, we used the software tpsRelw (Rohlf, 2003) to calculate a matrix of shape variables, called partial warp scores, which are coefficients indicating the position of an individual relative to the consensus shape, and further reduced them by a relative warps analysis (Zelditch et al., 2004). Relative warps analysis is a principal component analysis of the partial warps and uniform components (Zelditch et al., 2004). The

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calculated relative warp scores (RWS) were subsequently used as dependent variables in a MANOVA (Soto et al., 2008).

The possibility of detecting a relationship between fluctuating asymmetry and environmental stress is usually weak when testing only one trait (Leung et al., 2000). Thus, we obtained three measures of FA for our analysis. We defined wing size FA as the absolute difference between the right centroid size value and the left centroid size value (| R-L |) (Takahashi et al., 2011). We also defined wing venation FA as the absolute difference between the Euclidean distances in Procrustes coordinates between landmarks on the right and left radial and one of the anal wing veins (Smith et al., 1997; Takahashi et al., 2011). We confirmed that there was no significant directional asymmetry by performing a t-test on the signed right minus left trait values (all $P > 0.06$) and confirmed the absence of antisymmetry with tests of normality and visual inspection of data (all kurtosis > 1.00) (Palmer & Strobeck, 1986, 2003). We also confirmed no significant size dependence of FA by regressing the unsigned, absolute difference of the right and left trait values on trait size (all $P > 0.46$) (Palmer & Strobeck, 1986, 2003). The data were inspected for statistically significant outliers, defined as being greater than three standard deviations from the mean. Five individuals presenting significant aberrant values for life history traits, shape or size were excluded. We used the Kolmogorov-Smirnov test to check the distribution of data. All measures of FA, Procrustes distance and Mahalanobis distance were not normally distributed and we therefore log-transformed them before analyses though the log transformation did not normalize the data.

We used a linear mixed model approach to test for differences in FA, wing shape and wing size according to radiation applying PROC MIXED in SAS 9.1 (SAS Institute,

Cary, NC, USA). We separately tested three response variables (Procrustes distance, Mahalanobis distance and centroid size) one at a time as a function of sex (as fixed factors), pronotum length, radiation level and time to maturity (as covariates). In addition, we included in the model the interaction between radiation and sex, radiation and time to maturity and sex and time to maturity. We also included identity of the collection site and identity of the family of origin (nested within the collection site) as random effects to test the effect of both shared genes and developmental environment on the response variable. We used the Wald test (z) as the test of significance for our random effects. This is equivalent to the *F* test used to test significance of our fixed effects (SAS Institute Inc., 2008). We approximated degrees of freedom using the Satterthwaite approximation.

We also used a linear mixed model approach to test if within-family mortality predicted wing shape variation and FA within the family, while controlling for nymphal density, as indexed bythe total number of individuals per family. We tested average Procrustes distance per family, average Mahalanobis distance per family and average measures of shape variation per family, one at a time, as a function of radiation level and family mortality. We also included in the model the interaction between radiation level and mortality per family.

Additionally, we tested the independent effect of radiation on time to maturity, family mortality, family hatching success (measured as the number of individuals that hatched over total number of eggs produced), and fecundity (measured as the total number of egg pods produced) following the same linear mixed model approach. Because nymphal density varied during the rearing process, we also tested for independent effects of density on shape variation and FA within the family. For all linear mixed models, we

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applied an Akaike information criterion (AIC) approach for model selection, sequentially removing the least significant terms, starting from the interactions. We removed a term from the model only if removal did not increase the AIC values by more than 2.00 (Burnham & Anderson, 1998).

For the MANOVA, we applied PROC MIXED in SAS 9.1 and tested the relative warp scores as functions of the same fixed factors, covariates and random effects applied in the linear mixed model approach. We also tested the average relative warp scores per family as a function of radiation level, family mortality estimated as the percentage of a family that died and the interaction between radiation and mortality to determine if family mortality predicted shape variation within the family.

2.2. Results

2.2 a. Wing size

Radiation effects on wing size depended on whether the individual was a male or female (Table 2), with wing size increasing more with radiation levels in females (coefficient = 9.37×10^{-3} (3.51×10^{-3} SE), $t_{291} = 2.67$, $P < 0.01$) compared to males (coefficient = 1.07×10^{-2} (3.65×10^{-3} SE), $t_{299} = 2.94$, $P < 0.01$). In addition, males showed an increase in wing size with longer time to maturity (coefficient = 5.34×10^{-3} (1.54×10^{-3} SE), $t_{253} = 3.47$, $P < 0.01$), while time to maturity did not significantly affect wing size in females (coefficient = -1.85×10^{-3} (2.02×10^{-3} SE), $t_{363} = -0.92$, $P = 0.36$). Overall, the wing size response to radiation depended on the time at which the individual reached maturity (Fig. 3). Individuals that matured early had larger wings with increasing radiation levels while

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wing size in late maturing individuals decreased with increasing radiation levels (Table 2).

In addition, neither collection site ($z > 0.41$, $P > 0.26$) nor family ($z > 1.26$, $P > 0.10$) had a significant effect on wing size. Thus, the shared genes and environment were not significantly associated with variation in wing size amongst our experimental individuals.

2.2 b. Wing shape

We first analyzed wing shape, as indexed by either Procrustes distance (Table 3) or Mahalanobis distance (Table 4) as a function of radiation, sex, pronotum length and time to maturity. Radiation and time to maturity did not have joint or independent effects on any measure of wing shape (Tables 3 and 4). Males and females did not differ significantly in wing shape, nor did they differ significantly in how time to maturity was related to wing shape (Tables 3 and 4). Time to maturity was not significantly associated with shape of wings (Tables 3 and 4).

Males and females differed significantly in wing shape, as indexed by RWS, but sex did not have an interaction effect with radiation or time to maturity on wing shape. Radiation and time to maturity did not have interaction or independent relationships with wing shape (Table 5).

The collection site was not significantly related to wing shape when measured as Procrustes distance ($z > 0.00$, $P > 0.22$) or Mahalanobis distance ($z > 0.07$, $P > 0.23$). The

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relationship between collection site and wing shape variation when measured as relative warp scores was negligible. Additionally, the effect of family on shape was not significant when measured as Procrustes distance ($z > 0.10$, $P > 0.22$), Mahalanobis distance ($z > 0.86$, $P > 0.19$) or relative warp scores ($z > 0.27$, $P > 0.39$).

Radiation and family mortality were not jointly related to average shape as indexed by average Procrustes distance (coefficient = 4.25×10^{-6} (1.20×10^{-5} S.E.), $F_{97.5} = 0.13$, P = 0.72), average Mahalanobis distance (coefficient = 6.30×10^{-5} (3.22×10^{-4} S.E.), $F_{95.3} = 0.04$, P = 0.84) or average relative warp scores (coefficient = -3.05×10^{-6} (6.33×10^{-6} S.E.), $F_{299} = 0.23$, P = 0.63), nor was radiation significantly independently related to any measures of shape variation (P > 0.55). Average shape variation decreased significantly with increasing family mortality when measured as Mahalanobis distance (coefficient = -1.19×10^{2} (4.79×10^{-3} S.E.), $F_{97.7} = 6.18$, P = 0.01), but not as Procrustes distance (coefficient = -2.80×10^{-4} (1.20×10^{-5} S.E.), $F_{96.5} = 2.64$, P = 0.11) or relative warp scores(coefficient = 2.10×10^{-5} (5.30×10^{-5} S.E.), $F_{299} = 0.15$, P = 0.70).

2.2 c. Wing fluctuating asymmetry

Radiation, sex and time to maturity were not related to any measure of wing FA (Table 6). Collection site ($z > 0.14$, $P > 0.14$) was not significantly related to any measure of wing FA in addition to family not being significantly related to FA.

Average wing FA, as indexed by distance between landmarks on the radial vein decreased significantly with increasing family mortality (a: coefficient = -7.92×10^{-3} (3.72×10^{-2} S.E.), $t_{97.9} = -2.13$, $P = 0.04$; b: coefficient = -1.30×10^{-2} (4.84×10^{-3} S.E.), $t_{98} = -2.69$, $P = 0.01$) and when indexed by distance between landmarks on the anal vein

(c: coefficient = -4.95 x 10^{-3} (2.13 x 10^{-3} S.E.), t_{98} = -2.32, P = 0.02). Radiation was not, independently or jointly, significantly related to any measure of FA.

2.2 d. Life History

Radiation did not have an effect on time to maturity (coefficient = -9.57 x 10-3 (4.19 x 10^{-2} S.E.), $t_{18.3}$ = -0.23, P = 0.82). Family mortality decreased significantly with increasing radiation (coefficient = -4.45 x10^{-1} (1.64 x 10^{-1} S.E.), t_{117} = -2.71, P < 0.01) while family hatching success decreased with increasing radiation but not significantly (coefficient = -1.89 x 10^{-2} (1.02 x 10^{-2} S.E.), t_{112} = -1.85, P = 0.07). Additionally, radiation did not significantly affect fecundity (coefficient = 9.43 x 10-3 (9.82 x 10^{-3} S.E.), $t_{5.63}$ = 0.96, p = 0.38).

Nymphal density did not have a significant effect on the average wing size ($F_{93.4}$ = 0.00, P = 0.96), average wing shape as indexed by Procrustes distance ($F_{99.9}$ = 1.15, P = 0.29), Mahalanobis distance (F_{97} = 2.73, P =0.10), relative warp scores (F_{700} =0.02, P = 0.90) or any measure of wing FA (F > 0.01, $_{d.f.}$ > 94.9, P > 0.38).

2.3 Discussion

In this study of the trans-generational consequences of environmental stress we tested for effects of parental radiation exposure on developmental instability of grasshopper offspring. We used three measures of wing shape, a measure of wing size and various measures of wing fluctuating asymmetry to test if the degree of developmental instability was predicted by parental radiation exposure, and if that effect depended on individual

sex, body size and time to maturity. We also tested for effects of parental radiation exposure on family mortality, family hatching success and time to maturity in addition to whether mortality and nymphal density predicted developmental instability within families. If early developmental stages include more asymmetric individuals that suffer from differential mortality, this may bias estimates of the relationship between the environmental stressors, such as radiation, and asymmetry (Møller, 1997). Because we found weak, but non-significant effects of radiation on fecundity and hatching failure, we can exclude these factors as biasing our asymmetry estimates.

We also found that wing size, decreased relative to body size with longer maturation time and increasing parental radiation exposure. Thus, with increasing time to maturity, late maturing individuals born to parents from high radiation sites were smaller than late maturing individuals whose parents came from low radiation sites. Males responded to increasing parental radiation exposure with increasing wing size while wing size decreased, but not significantly, in females. Radiation exposure did not influence wing fluctuating asymmetry, independently or in combination with any other variable. Previous studies on Orthopteran growth and development show that environmental variation significantly affects the plasticity of adult size and time to eclosion (Moore, 1948; Willott & Hassall, 1998; Finch et al., 2008). Additionally, this effect is greatly influenced by developmental stage. Individuals raised under optimal environmental conditions during early nymphal stages tend to reach adulthood with fewer molts and at a larger size compared to individuals raised under sub-optimal conditions regardless of whether conditions are reversed at later nymphal stages (Flanagin et al., 2000; Miller et al., 2001; Taniguchi & Tomioka, 2003). This suggests that the physiological and

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hormonal events necessary for the timing of eclosion and adult size are already fixed by the later stages (Bradshaw & Johnson, 1995). Thus, if radiation is interfering with these physiological events early in development, it may be reflected in smaller adult size and delayed eclosion.

For example, the interaction effect of radiation exposure and longer maturation time resulting in smaller size at maturity may suggest that the offspring's physiological ability to metabolize and allocate nutrients towards growth is developmentally unstable as a result of increasing parental radiation exposure. In Orthopteran species, body size is significantly correlated with fitness components. For example, Landa (1992) found that late hatching nymphs of *Arphia sulphurea* and *Chortophaga viridifasciata* were smaller, suffered higher overwinter mortality and reached reproductive maturity later compared to controls. Simmons (1995) found in the field cricket (*Gryllus campestris*) that larger males, as measured by their body length, were more successful in obtaining mates as a result of possessing a larger harp which in turn affected the carrier frequency of the male's courtship song. In studies on bush crickets, larger males tend to produce more ejaculate compared to smaller males (Wedell, 1997). Additionally, larger size has been shown to contribute to an individual's resistance to parasitism (Danyk et al., 2000) is a determinant of fecundity in females (Fox and Czesak, 2000). Previous studies have demonstrated a physiological trade-off between developmental rate and size at maturity for various grasshopper species in response to geographical and seasonal variation in food quality (Asshoff & Hattenschwiler, 2005; Fielding et al., 2007; Jannot et al., 2009) that appears to occur at the metabolic level (Behmer & Elias, 1999; Hahn, 2005). Møller & Mousseau (2011) have suggested that the difference in susceptibility to radiation

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among invertebrates may in part be due to these differences in metabolic rates relative to body size. Species with relatively high metabolic rates may be more vulnerable to radiation effects due to increased demand for antioxidant defense (Møller & Mousseau, 2011). If this is true also at the intra-specific level, faster development may increase susceptibility to oxidative stress and compromise the metabolic capabilities and allocation of resources for further development.

Additionally, poor maternal contribution to eggs as a consequence of parental radiation exposure may be exacerbating developmental selection pressure. During oogenesis, females allocate resources including carbohydrates, proteins, lipids and water to eggs (Wheeler, 1996). Grasshoppers obtain nutrients for oogenesis during adult feeding and insufficient nourishment can adversely affect egg production and subsequent offspring development (Bellmann, 1985; Wheeler, 1996; Fox & Czesak, 2000). Egg size has been positively correlated with offspring survivorship and size in unfavorable environments (Braby, 1994; Mousseau & Fox, 1998). Mothers exposed to persistent oxidative stress may incur metabolic constraints that limit the acquisition of resources for eggs and decrease the phenotypic quality of their offspring. Thus under conditions when mothers are limited in the availability of important nutrients for eggs, as might be the case in radioactively contaminated areas, offspring are more vulnerable to environmental perturbations, experience greater developmental selection pressure and may need to compensate for reduced egg resources with longer development time.

The negative trend between family mortality and FA within families suggests that more asymmetric individuals suffered higher mortality rates compared to symmetrical individuals. Considering that offspring are often produced in high numbers, one would

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expect a decrease in overall FA with increasing mortality as developmentally unstable individuals are removed by selection. This is especially so under chronic environmental stress where developmental selection becomes more intense (Møller, 1997). Increased environmental stress may interfere with physiological mechanisms involved in maturation. For example, studies on insect ecdysis (i.e. molt) found that failure of ecdysis appears to occur due to disruption of muscle contractions that are involved in working the individual forward out of the old cuticle (Hughes, 1980; Ewer, 1997; Park et al., 2003). Irradiation experiments found ecdysis failure to occur due to abnormal development of abdominal structures responsible for expanding the abdomen and breaking the old cuticle, similar to results found in insects treated with natural or synthetic juvenile hormone analogs (Reynolds, 1980). Under these conditions, adult eclosion failure was found to increase with prolonged exposure to radiation (Beard, 1972; Sharma & Dwivedi, 1997; Faruki et al., 2007). In one study of the tobacco hornworm *Manduca sexta*, duration of development in offspring of irradiated parents was roughly 10% longer and less than 60% emerged as adults compared to 90% of controls (Seth & Reynolds, 1993). If families are experiencing increased genomic instability due to parental radiation exposure, they may experience greater selection pressure due to the increased number of phenodeviants. If such deviance is reflected in physiological, immunological and behavioral function of the individual, insects may be particularly vulnerable to developmental selection pressure from parental radiation exposure because malformed or physiologically deviant individuals would be unable to successfully eclose into adult form.

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In conclusion, the effect of parental radiation exposure can adversely affect offspring development and fitness depending on developmental trajectories. However, caution in interpreting results is required. It is not certain whether this effect was due to transgenerational genomic instability or other influences such as maternal contribution to eggs. We must also consider the possibility that the levels of radiation assessed in this study were not sufficient for affecting FA in our model species. In order to further assess the correlation of FA with fitness, future studies may wish to include more direct fitness parameters such as a female's lifetime contribution to the next generation in addition to life history traits such as escape behavior, mating success, immunocompetence and maternal investment in egg content. Including more replication in the experimental design so that the repeatability of such measures can be assessed will help further our understanding of the relationship between FA and fitness.

Table 1 Number of families and average number of individuals included in our study in each collection site. See Material and Methods for more details.

Site	Radiation (μSv/h)	No. families	Average number of F1 progeny per family (minimum-maximum)
Chystohalivka(1)	0.03	10	10.8 (0-16)
Rozizhdzhe (2)	0.1	29	9.3 (0-17)
Vesniane (3)	5.4	38	11.7 (5-20)
Krasnytsia (4)	10.72	21	14 (4-26)
Red Forest 1(5)	35.31	21	12.6 (5-27
Red Forest 2 (6)	50.06	4	16.5 (7-23)

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Table 2 Results for linear mixed model for wing size variation as measured by centroid size. Statistically significant relationships ($P < 0.05$) are marked in bold.

Final Model	Coefficient	S.E.	d.f.	F	P
Pronotum	7.11×10^{-3}	2.66×10^{-3}	1, 414	7.14	**< 0.001**
Radiation	9.37×10^{-3}	3.51×10^{-3}	1, 291	7.95	**0.01**
Time to Maturity	-1.85×10^{-3}	2.02×10^{-3}	1, 363	1.37	0.24
Sex			1, 404	11.43	**< 0.001**
Time to Maturity x Radiation			1, 319	7.44	**0.01**
Radiation x Sex			1, 401	4.41	**0.04**
Time to Maturity x Sex			1, 404	12.92	**< 0.001**

Table 3 Linear mixed models for shape variation as measured by Procrustes distance. Akaike information criterion (AIC) model selection approach removes least significant terms starting with interactions. Dropped terms are reported in the order with which they were removed from the model, with their associated statistics at removal.

Final Model	Coefficient	S.E.	d.f.	F	P	AIC
Pronotum	-3.50×10^{-4}	1.37×10^{-2}	1, 411	0.01	0.98	764.0
Time to Maturity	-7.60×10^{-3}	5.09×10^{-3}	1, 201	2.23	0.14	
Radiation	-1.40×10^{-3}	2.14×10^{-3}	1, 9.1	0.43	0.53	
Sex	-	^{2}	1, 415	2.39	0.12	
Dropped Terms						
Radiation x Sex			1, 411	0.11	0.74	791.1
Time to Maturity x Sex			1, 410	1.08	0.30	781.7
Time to Maturity x Radiation			1, 248	2.98	0.09	775.5

Table 4 Linear mixed models for shape variation as measured by Mahalanobis distance. Akaike information criterion (AIC) model selection approach removes least significant terms starting with interactions; statistically significant relationships ($P < 0.05$) are marked in bold. Dropped terms are reported in the order with which they were removed from the model, with their associated statistics at removal.

Final Model	Coefficient	S.E.	d.f.	F	P	AIC
Time to Maturity	-5.07×10^{-3}	4.57×10^{-3}	1, 248	1.23	0.27	654.20
Sex			1, 416	5.17	**0.02**	
Radiation	-8.90×10^{-4}	1.96×10^{-3}	1, 10.6	0.20	0.66	
Pronotum	-2.48×10^{-3}	1.20×10^{-2}	1, 417	0.04	0.84	
Dropped Terms						
Days to Maturity x Sex			1, 407	0.48	0.49	683.00
Radiation x Sex			1, 407	0.59	0.44	675.90
Days to Maturity x Radiation			1, 305	2.17	0.14	666.60

Table 5 Results for multivariate mixed model for wing shape variation as measured by Relative Warp Scores.   Akaike information criterion (AIC) model selection approach removes least significant terms starting with interactions; statistically significant relationships ($P < 0.05$) are marked in bold. Dropped terms are reported in the order with which they were removed from the model, with their associated statistics at removal.

Final Model	Coefficient	S.E.	d.f.	*F*	*P*	AIC
Pronotum	7.00×10^{-4}	4.02×10^{-4}	1, 3300	3.04	0.08	-10029.9
Radiation	2.00×10^{-5}	5.70×10^{-5}	1, 3300	0.17	0.68	
Time to Maturity	2.82×10^{-4}	5.70×10^{-5}	1, 3300	2.77	0.10	
Sex			2, 3300	3.06	**0.05**	
Dropped Terms						
Radiation x Sex			1, 3297	0.07	0.79	-10025.1
Time to Maturity x Radiation			1, 3298	0.58	0.45	-10027.0
Time to Maturity x Sex			1, 3299	0.55	0.46	-10028.5

Table 6 Results for linear mixed model for wing shape FA as measured by distances between landmarks on the radial (LM1-LM2, LM2-LM3) and anal (LM5-LM6) veins. Akaike information criterion (AIC) model selection approach removes least significant terms starting with interactions. Dropped terms are reported in the order with which they were removed from the model, with their associated statistics at removal.

Final Model	Coefficient	S.E.	d.f.	F	P	AIC
Radial Vein Distance (LM 1 - LM 2)						
Time to Maturity	-8.08×10^{-3}	1.45×10^{-2}	1, 336	0.31	0.58	1632.80
Sex			1, 413	0.95	0.33	
Radiation	-8.38×10^{-1}	5.36×10^{-3}	1,11.6	2.44	0.14	
Pronotum	-2.70×10^{-2}	3.98×10^{-2}	1, 414	0.46	0.50	
Dropped Terms						
Radiation x Sex			1, 411	0.00	0.95	1657.40
Days to Maturity x Radiation			1, 397	0.05	0.82	1649.90
Days to Maturity x Sex			1, 413	0.22	0.64	1637.80
Radial Vein Distance (LM 2 - LM 3)						
Time to Maturity	-1.09×10^{-2}	1.36×10^{-2}	1, 207	0.64	0.42	1598.80
Pronotum	-1.17×10^{-2}	3.08×10^{-2}	1, 415	0.14	0.12	
Radiation	-7.43×10^{-3}	4.63×10^{-3}	1, 46	2.57	0.70	
Dropped Terms						
Radiation x Sex			1, 410	0.09	0.77	1625.00
Days to Maturity x Sex			1, 408	0.14	0.71	1617.60
Days to Maturity x Radiation			1, 214	0.09	0.76	1612.50
Sex			1, 412	0.00	0.96	1600.20
Anal Vein Distance (LM 5 - LM 6)						
Time to Maturity	1.28×10^{-3}	9.15×10^{-3}	1, 218	0.02	0.89	1266.60
Radiation	1.64×10^{-3}	3.11×10^{-3}	1,50.1	0.28	0.60	
Pronotum	3.96×10^{-2}	2.06×10^{-2}	1, 415	3.69	0.06	
Dropped Terms						
Days to Maturity x Sex			1, 408	0.58	0.45	1294.10
Radiation x Sex			1, 411	0.83	0.36	1288.60
Days to Maturity x Radiation			1, 226	0.77	0.38	1281.20
Sex			1, 412	0.02	0.90	1268.80

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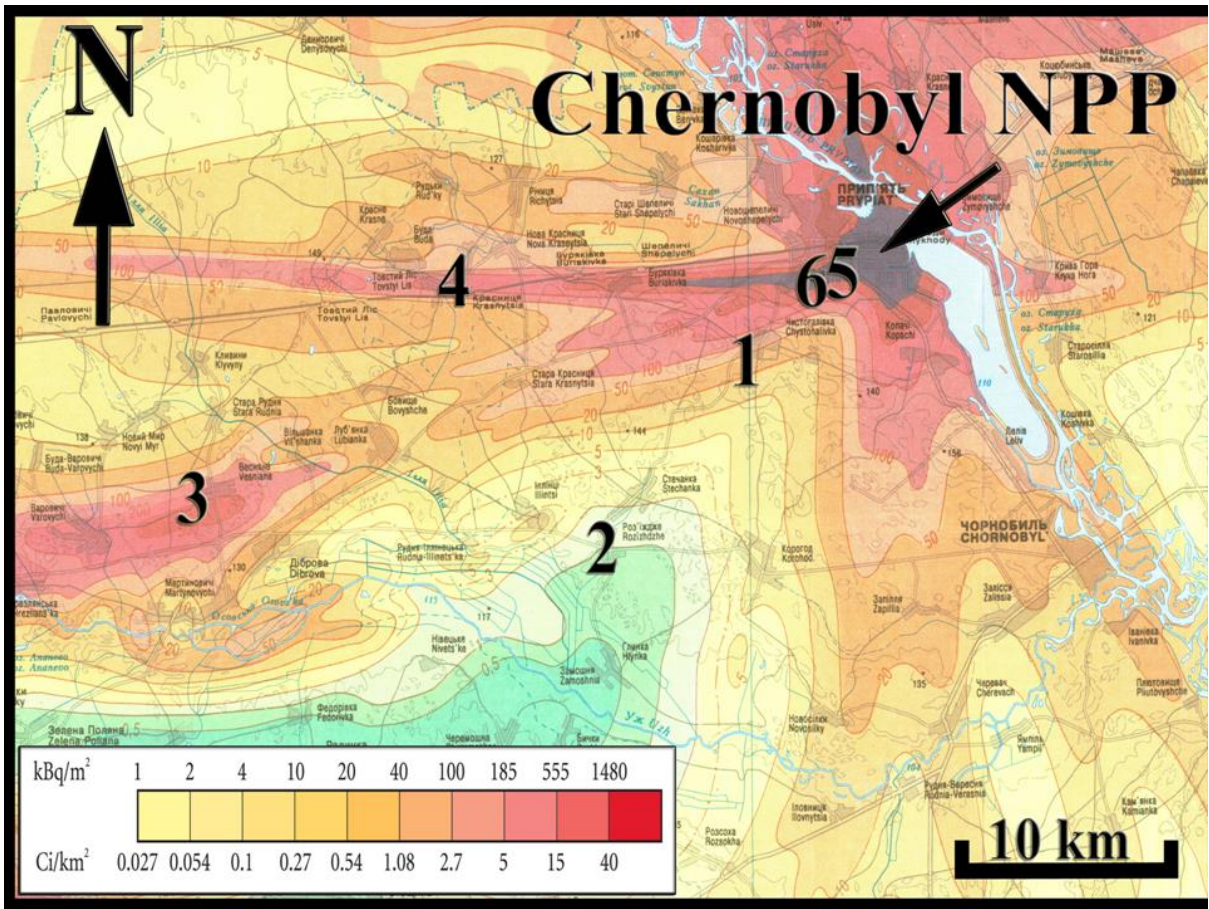
Figure 2.1. Locations of field collection sites and background radiation around Chernobyl

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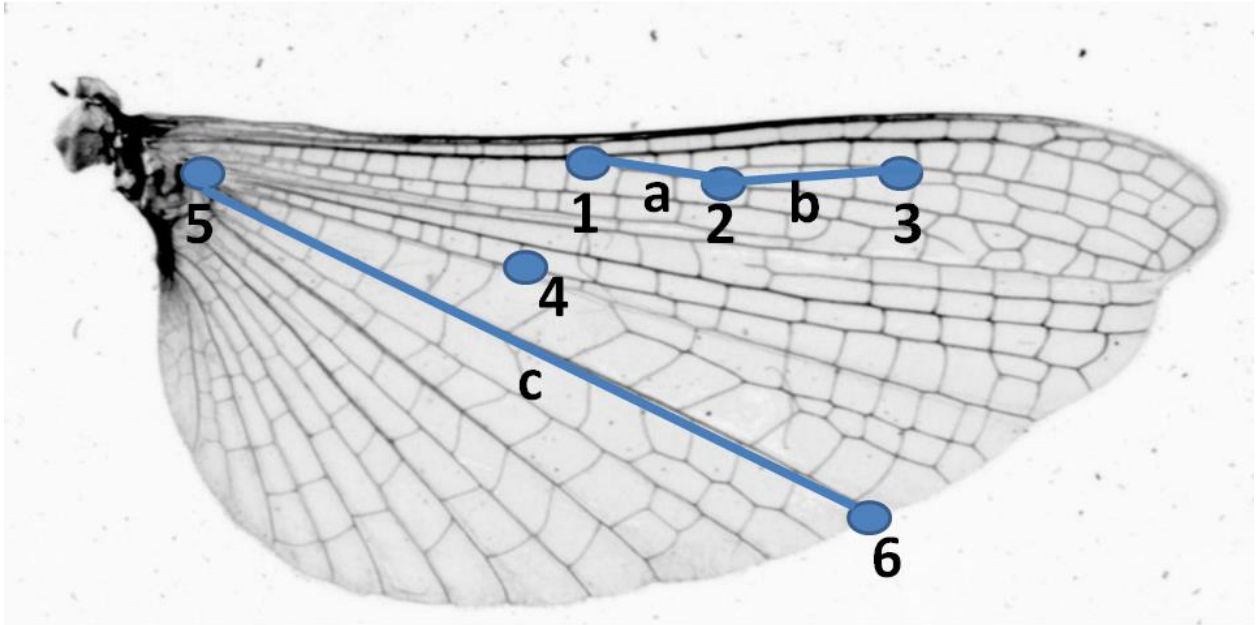
Figure 2.2 Hind-wing of lesser marsh grasshopper (*C. albomarginatus*) showing landmark positions on wing veins (numbers) and vein lengths (letters) selected for fluctuating asymmetry measurements with image analysis
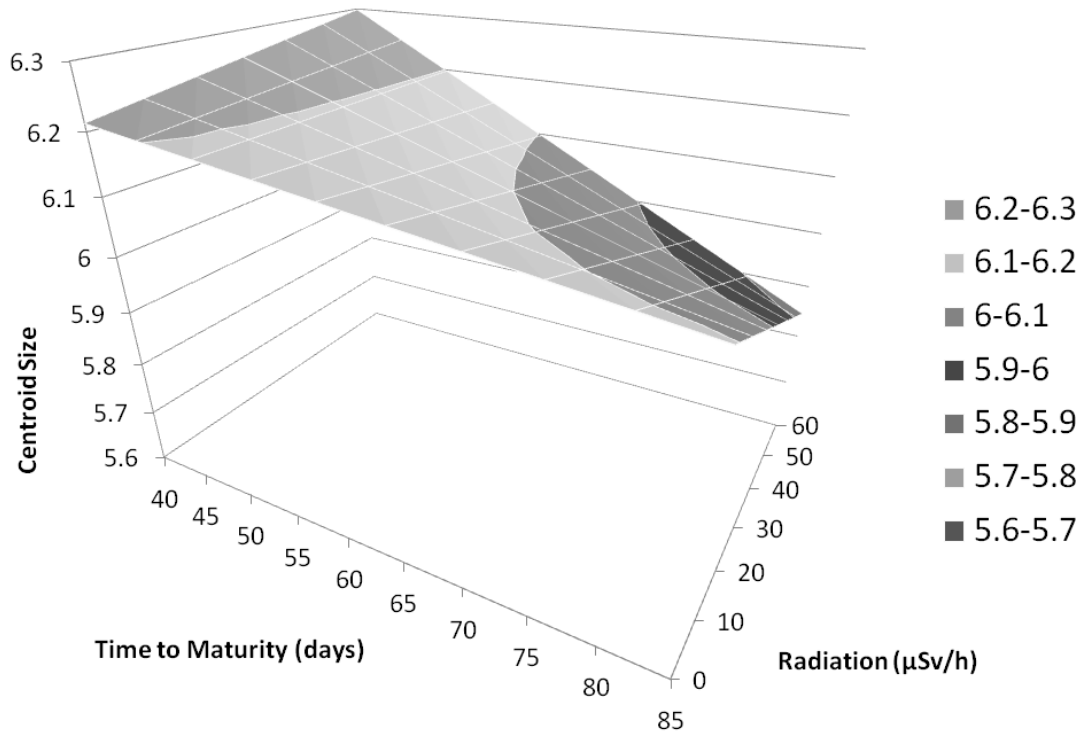
Figure 2.3 Relationship between centroid size, parental radiation exposure and time to maturity. Results for both males and females were combined because they showed a similar pattern. A surface was interpolated based on the coefficients for the effects of radiation, time to maturity and the interaction between the two in the best-fit model

CHAPTER 3

THIRTEEN-YEAR CICADAS IN SOUTH CAROLINA: USING MULTI-MEDIA AND
THE NATIONAL ATLAS TO EXAMINE INFREQUENT HIGH MAGNITUDE
ECOLOGICAL PHENOMENA[3]

Introduction

Large, infrequent disturbances (LIDs), such as harmful algae blooms, epidemics, biological invasions, natural and human-caused disasters, can have global and devastating consequences for human populations and ecological processes with lasting and cascading effects (Easterling et al., 2000). As the variability of extreme climate conditions (Parmesan, 2006) and anthropogenic influence on ecosytems increase (Nelson et al., 2006), tracking the effects of these large-scale and sporadic events immediately after the disturbance occurs is critical for identifying factors that will influence long-term ecological functioning, such as landscape structuring, nutrient cycling, and species abundance and distribution (Lindenmayer et al., 2010).

However, monitoring spatial and temporal patterns of LIDs is costly, and the sporadic nature of these events makes it logistically difficult to mobilize necessary resources to evaluate patterns as they occur (Carr, 2004). Additionally, the origin and physical characteristics of the disturbance can affect the spatial and temporal pattern of

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[3] Beasley, D.E., Waldron, J.L., Welch, S.M. Benson, E.P. and Mousseau, T.A. Submitted to *BioScience* 2/26/2013.

the ecological response (Dale et al., 1998). For example, natural disturbances (e.g., floods and fire) occur over an extended period, while wind-driven events (e.g., tornados and hurricanes) occur within a short time frame with varying degrees of intensity. Anthropogenic disturbances, such as oil spills and nuclear accidents, can affect broad areas with consequences lasting decades (Foster et al., 1998). The interacting effects of both natural and anthropogenic disturbances can also influence the pattern of ecological responses in an area. For example, Howard (2012) demonstrated that the combination of storm surge flooding from Hurricane Katrina and slow drainage due to levee construction resulted in increased mortality of native trees in a bottomland hardwood forest and the spread of the invasive Chinese tallow tree (*Triadica sebifera*). The unforeseen consequence of the levees led to a dramatic change in the plant community composition and a significant loss in biodiversity. Similarly, Nelson et al. (2009) detected significant shifts in vegetation dynamics in forested bottomland conservation areas in response to management practices and tornado disturbance history.

Given the unpredictability of LIDs, it is extraordinarily difficult to monitor their immediate impact. The use of citizen volunteers might help capture early, important ecological signals immediately following LIDs for the purpose of providing real-time data for conservation management practices and policy (Conrad and Hilchey, 2011). Community-based monitoring has been used extensively for decades, particularly in developing countries where financial resources are limited (Danielsen et al., 2005). Recently, broader availability of the Internet and other technologies has increased opportunities and potential effectiveness of citizen science as a research tool for general, hypothesis driven ecological studies (Newman et al., 2012; Worthington JP et al., 2012).

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Additionally, volunteers are typically invested in monitoring the local environment and have the added advantage of being on- or near-site when the event occurs (Carr, 2004; Beasley et al., 2012). Past projects that used citizen volunteers generated large and information-rich datasets and demonstrated that citizen-based data can be just a reliable as data collected by professionals (Delaney et al., 2008; Silvertown, 2009); however, the use of citizen science data for ecological research can be challenging due to difficulties in determining how well citizen-science data reflect biological realities and identifying biases in human demographics (Gallo & Waitt, 2011; Miller et al., 2012).

The spring 2011 emergence of the Brood XIX periodical cicada (*Hemiptera: Cicadidae: Magicicada*) provided a unique opportunity to assess the effectiveness of citizen scientists in monitoring a LID event and identify possible bias from human demographics. Brood XIX consists of three species (*M. tredecassini, M. tredecim, M. tredecula*) that historically ranged along the east coast and Midwestern United States. Every 13 years, final instars emerge synchronously from the ground and eclose into adult forms, living approximately 4-6 weeks (Williams and Simon, 1995). Males climb into the forest canopy and produce a species-specific mating chorus that, in large aggregations, can range from 50 to 80 decibels (Williams and Smith, 1991). Historically, large eruptions were closely associated with hardwood-dominated forests, with lower relative densities occurring in conifer-dominated forests (White, 1982); however, increased habitat fragmentation due to human activity and global climate change has significantly reduced cicada populations, with some broods going locally extinct (Cooley et al., 2004). We assumed that the range of Brood XIX spanned across the Piedmont region in South Carolina, based on anecdotal accounts (Beasley et al., 2012).

The goal of this study was to examine both the ecological and socio-demographic factors in relation to the spatial distribution of citizen reports of cicada occurrence. Specifically, we aimed to determine if the availability of hardwood forests and human activity on the landscape explained the spatial distribution of citizen reports. Female cicadas are known to successfully lay eggs in the branches of both woody tree species and conifer tree species (Williams and Simon, 1995). However, hatching success is significantly reduced in conifer trees due to increased resin production that seals the eggs in the branch and prevents nymphs from dropping to the soil (White, 1982). Once in the ground, cicada nymphs feed exclusively on tree root xylem throughout the juvenile stage of their development, showing very little movement once they begin parasitizing a root system (Karban, 1980). Based on this strong dependency on the tree host and the prominence of the timber industry in the South Carolina Piedmont, where approximately seven percent of the land cover is devoted to timber harvesting and pine plantations (US Geological Survey, 2011), we expected the citizen reports to reflect the cicada's sensitivity to changes in habitat structure and availability.

Conversely, specific community characteristics could also influence the distribution of reports. For instance, studies have demonstrated that socio-demographic characteristics such as education, knowledge and use of an environmental resource in question, play a significant role in an individual's attitude towards the environment and their willingness to protect it (Hunter et al., 2012). Thus, these same socio-demographic factors may also influence an individual's likelihood to participate in an environmental monitoring project, especially if there's a perceived risk of harm to a valuable resource. In the case of cicada emergences, large aggregations can potentially kill young trees and

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cause flagging in older trees as a consequence of oviposition behavior (Miller &

Crowley, 1990).

Overall, our study will assess the potential promise and challenges in using citizen

reports to capture early ecological signals following a LID event.

Methods

Study area

The South Carolina Piedmont includes 23 counties, consisting of one third of the

state, making it the largest physiographic province in South Carolina. The landscape is

dominated by agriculture and managed woodlands, which consist of hardwood (*Quercus

sp., Liquidambar styraciflua, Carpinus caroliniana, Ulmus alata*), pine (*Pinus taeda*) and

mixed pine-hardwood forest stands. Oak-hickory forests are characteristic of the

piedmont region, occurring in fragmented stands throughout the region. Human activity,

such as farming, urban development and the introduction of loblolly pine (*P. taeda*) as a

cash crop has significantly altered the vegetation of the region and the availability of

suitable habitat for native species (SCDNR report; Fig. 1).

Data collection

We followed the sampling protocol outlined in Beasley et al. (2012).Three South

Carolina state institutions established web sites for citizen scientists to send reports of

periodical cicada sightings one month prior to the anticipated emergence season in

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Spring, 2011. We used local public media that reached areas in the Carolinas and Georgia to inform citizens of the web sites in addition to correspondence with professional organizations. We asked volunteers to report specific locations of sightings in the form of address, street intersections, GPS coordinates and county as well as contact information for follow-up questions. Citizen volunteers validated reports by submitting sound and/or visual documentation. Additionally, we visited site locations to confirm *Magicicada* presence. We entered report coordinates into a Geographical Information System (GIS) to map spatial distribution.

We collected socio-demographic data from the United States Census Bureau website (2012). We expected that areas with a higher relative density of primary education-aged children were more likely to participate in the study due to growing interest in including students in relevant environmental studies to improve science literacy and appreciation (Dvornish et al., 1995; Hurd, 1998). Furthermore, we expected that areas with more individuals with a college degree or higher would be more likely to participate in the study due to a greater interest in environmental responsibility (Kim et al., 2012). We assumed that areas with more individuals working in natural resources and/or farming are more likely to report due to invested interest in determining the risk of the eruption on work-related concerns. Lastly, we also assessed the role of residency status in distribution of reports with the expectation that individuals long lived within a community are more likely to give a report due to an interest in events that impact community well-being.

We also collected land cover data from the USGS GAP Analysis Program for 23 counties and 154 sub counties in the Piedmont region of South Carolina (US Geological

Survey, 2011). The GAP land cover database is based on the NatureServe Ecological Systems Classification and provides the most consistent data on vegetative land cover. Additionally, it is recommended for studies that wish to include wildlife habitat and land use information in conservation management plans (US Geological Survey, 2011). We used a multiple-scaled approach to characterize land cover ranging from coarse (1) to fine (3) scales to account for the patchy availability of cicada habitat (Table 1).

Statistical analysis

We examined the spatial distribution of citizen reports at both county and sub-county levels to capture variation in distribution at coarse and fine spatial scales. We used a total of 11 *a priori* candidate models that reflected cicada ecology and community socio-demographics respectively (Table 2). Five *a priori* candidate models were based on cicada habitat availability as indicated by percentage of hardwood tree stand land cover, temperate forest land cover, disturbed/modified land cover and percentage of land cover devoted to pine plantations and timber harvest activities. The remaining six *a priori* candidate models reflected a county or sub-county's socio-demographics in regards to population density, percentage of the population with a bachelor's degree or higher, percentage of families with school-aged children, percentage of population born in-state and the percentage of the population that works outdoors and in agricultural or forestry industries.

We checked for co-linearity between our predictor variables by comparing the variance inflation factor (VIF) for each variable using PROC REG in SAS 9.3 (SAS Institute, Cary, NC, USA). VIF values of ten or above suggest that a predictor variable is

highly correlated with other variables in the model and may be redundant (Kutner et al., 2004).

We compared *a priori* models using Akaike's Information Criterion (AIC) and examined goodness-of-fit by comparing ΔAIC values to the most heavily parameterized model, referred to from this point on as the global model (Burnham and Anderson, 2002). At the county level, we used a generalized linear model approach to test the relationship between predictor variables and the number of reports in an area using PROC GENMOD in SAS 9.3 (SAS Institute, Cary, NC, USA). For the sub-county level, we converted our response variable to presence/absence data and applied a binomial logistic regression analysis using PROC LOGISTIC to account for the sporadic nature of the distribution of reports at the smaller scale

Results

The web sites received a total of 191 reports with 19 South Carolina piedmont counties reporting sightings. We did not detect significant co-linearity between our socio-demographic predictor variables as indicated by our VIF value being less than 5.49 (Table 2), nor did we detect a significant correlation between our ecological predictor variables with VIF values less than 6.20 (Table 3). Thus, we concluded that there was no redundancy in our candidate models and retained all of our predictors in the global model for comparison.

At the county level, the distribution of reports increased significantly in relation to areas with more hardwood tree land cover, which more directly relates to cicada habitat preference at a fine landscape scale. At a broader land scape scale, the distribution of

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reports significantly decreases in areas with more temperate forest land cover. Similarly, at a broad landscape scale, the distribution of reports increased in areas that had undergone land clearing and modification. Timber harvest activity and pine plantations did not significantly relate to the spatial distribution of reports.

When analyzing socio-demographic predictor variables at the county scale, the distribution of reports significantly increased with the percentage of families with school-aged children. We did not detect a significant relationship from the other socio-demographic factors (Table 5).

At the sub-county scale, the distribution of reports significantly increased in relation to areas with more pine plantations and areas with more hardwood tree land cover. We failed to detect a significant relationship from the remaining ecological and socio-demographic predictor variables (Table 6).

Discussion

Our study aimed to assess the spatial distribution of citizen reports on periodical cicada emergences in relation to known ecological landscape factors that influence cicada behavior and community socio-demographics to determine the potential role of citizen reports in tracking a LID event. Overall, we found that fine scale measures of ecological landscape factors such as hardwood tree cover and planted pine plantations significantly related to the increase in distribution of citizen reports while larger scale measures such as temperate forest and modified landscape significantly related to a decrease in distribution of reports. The only socio-demographic factor that significantly related to the

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distribution of reports was percentage of families with primary school aged children in an area, with the distribution increasing with increasing families.

The increase in the distribution of reports in relation to hardwood tree land cover and planted pine stands suggests that citizen reports can reflect the adaptability of periodical cicadas to changing landscape and thus ecological processes at a relatively fine scale. We expected to see a higher distribution of reports in areas with more hardwoods given the species dependence on the tree host for oviposition and juvenile development. Additionally, there is documented evidence of periodical cicada emergences occurring in pine plantations and orchards, particularly if hardwood trees were present in the area prior to the establishment of the plantation. Cicada nymphs can feed successfully on conifer roots (Lloyd and White, 1987) and thus emergences can occur though success of the next generation will be greatly reduced due to the low hatching success from conifer branches (White, 1982). Comparatively, coarse landscape scales showed an inverse relationship with the distribution of reports suggesting citizen reports may be confounded by the availability of reporters in remote areas such as temperate forests and cleared landscapes.

Our finding of increased distribution of reports being significantly related to percentage of families with primary school aged children provides a means of improving participation in environmental monitoring projects. For instance, we did not receive reports from some areas where we know cicada emergences occurred, suggesting participation in the study was not evenly spread across the population. Hobbes and White (2012) found that individuals from socio-economic deprived areas were underrepresented in environmental monitoring programs at both the national and local levels and it may

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have been a contributing factor in our study as 17 percent of the population in South Carolina has income below the poverty line (U.S. Census, 2012). The advantage of using public school systems for citizen science projects is in disseminating information more efficiently throughout the community, fostering community action and increasing awareness of environmental concerns (Thornton and Leahy, 2010). Previous citizen science projects on species distribution and natural resource management successfully used participants from public schools (Dvornich et al, 1995; Weckel et al., 2010).

By taking advantage of established community institutions that improve citizen participation and provide an assessable interface for data collection, citizen reports can more effectively provide information about ecological responses to LID events such as hurricanes and wildfires (Foster et al., 1998). For LID events with low temporal predictability such as tornados, the use of citizen reports may still be applicable if data collection occurs consistently over time with a target area in mind. For example, Jaramillo et al. (2012) took advantage of ecological surveys conducted weeks before and after Chile's 2010 8.8 magnitude earthquake to assess the coastal ecosystem's response to major earthquakes in relation to human land-use, species mobility and shore type.

In conclusion, with the increasing awareness of the growing influence of human activity on ecosystem integrity and the desire to integrate public interest with authentic scientific research (Pretty, 2003; Dickinson et al., 2012), there is potential for the use of citizen science to be a successful tool for monitoring LID events and detecting important ecological signals. Our study had the advantage of tracking a very charismatic species in appearance, sound and number, increasing the likelihood of citizens sending in reports and accurately identifying the species though how much these reports reflect ecological

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responses to LIDs may be limited by the scale at which the data is analyzed. Specifically, the data collected in our study will provide a starting point for assessing how changing tree host composition due to human activity on the landscape in the Piedmont will impact the distribution of the periodical cicada over time. To increase the reliability and success of applying citizen science in tracking ecological responses to LIDs, future studies must first establish an assessable interface for data collection and maintain sufficient participation through collaboration with local community institutions, such as public schools (Cooper et al., 2007; Hochachka et al., 2012). Such collaboration has the promise of improving long-term social and scientific networks between research institutions and local communities.

Table 1: Predictor variables used to evaluate spatial distribution of citizen reports of cicada occurrence in the Piedmont physiographic province of South Carolina.

Predictor Variables	Description	Source
Population Density	Population density for individuals 25 years of age and older	U.S. Census Bureau, 2012
Education Level	Percentage of population with a bachelor's degree or higher	
School-Aged Children	Percentage of families with children between the ages of 1 and 17 years of age	
Resident	Percentage of population born in South Carolina	
Occupation	Percentage of population involved in construction and natural resource management	
Industry	Percentage of population involved in agricultural and forestry industries	
Disturb1	Recently disturbed/modified land use as defined as land clearing at the coarse scale	U.S. Geological Survey, 2011
Tempfor1	Percentage of land covered by temperate forest at the coarse scale	
Forestry3	Percentage of land covered by forest used for timber harvest at the fine scale	
Plant_pine3	Percentage of land covered by planted pine forest at the fine scale	
Hardwood3	Percentage of land covered by hardwood forest at fine scale	

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Table 2: *A priori* candidate models used for citizen report occurrence in relation to ecological and socio-demographic variables. Model type, name, structure and total parameters (K) are provided.

Model Type	Model Name	Model Structure	K
Socio-demographic			
	Population density	pop_density	1
	Higher education	%_BD_higher	1
	School children	%_children	1
	State resident	%_resident	1
	Outdoor occupation	%_occupation	1
	Agricultural/Forestry Industry	%_industry	1
Ecological			
	Landscape disturbance	%_disturb1	1
	Temperate Forest	%_tempfor1	1
	Timber Harvest	%_forestry3	1
	Planted Pines	%_plant_pine3	1
	Hardwood trees	%_hardwood3	1
Global	Ecological/Socio-demographic	%_hardwood3+%_disturb1+%_forestry3+%_tempfor1+%_plant_pine3+pop_density+%_BD_high+%_children+%_resident+%_occupation+%_industry	11

Table 3: Co-linearity between socio-demographic predictor variables. Variance inflation factor (VIF) less than ten indicates a non-significant correlation.

Predictor Variables	Model Code	VIF
Population Density	Pop_Density	5.19
Education Level	%_BD_Higher	4.68
School-Aged Children	%_Children_U6_B6_17	1.86
Resident	%_Resident	1.79
Occupation	%_Occupation	5.49
Industry	%_Industry	3.60

Table 4: Co-linearity between ecological predictor variables. Variance inflation factor (VIF) less than ten indicates a non-significant correlation.

Predictor Variables	Model Code	VIF
Disturb1	%_Disturb1	5.82
Tempfor1	%_Tempfor	2.41
Forestry3	%_Forestry3	3.14
Plant_pine3	%_Plant_Pine3	2.98
Hardwood3	%_Hardwood3	6.20

Table 5: Candidate models (county) ranked according to AIC model selection. Models are ranked in order of support.

Order	Model	Scale	AIC	Δ AIC	Beta coefficients	Standard Error	p value
1	Global Model		109.5637	0			
2	%_ Tempfor	1	204.499	94.9353	-4.23×10^{-2}	9.4×10^{-3}	**< 0.01**
3	%_Children_U6_B6_17	1	204.5269	94.9632	4.57×10^{-1}	1.15×10^{-1}	**< 0.01**
4	%_ Hardwood3	3	206.1182	96.5545	1.33	3.25×10^{-1}	**< 0.01**
5	%_ Disturb1	1	219.1062	109.5425	2.02	9.79×10^{-1}	**0.04**
6	%_ Forestry3	3	221.7741	112.2104	2.62	1.88	0.16
7	%_BD_Higher	1	221.9247	112.361	1.85×10^{-2}	1.34×10^{-2}	0.17
8	% Plant_Pine3	3	223.3665	113.8028	1.36×10^{-1}	2.18×10^{-1}	0.53
9	%_Resident	1	223.4425	113.8788	-4.30×10^{-3}	7.8×10^{-3}	0.58
10	Pop_Density	1	223.589	114.0253	1.0×10^{-3}	2.5×10^{-3}	0.69
11	%_Occupation	1	223.7476	114.1839	-1.4×10^{-3}	4.41×10^{-2}	0.98
12	%_Industry	1	233.7111	124.1474	2.0×10^{-3}	1.02×10^{-2}	0.85

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Table 6: Candidate models (sub-county) ranked according to AIC model selection. Models are ranked in order of support.

Order	Model	Scale	AIC	Δ AIC	Beta coefficients	Standard Error	p value
1	Global Model		174.818	0			
2	%_Plant_Pine3	3	178.561	3.743	1.27	4.12×10^{-1}	**< 0.01**
3	%_Disturb1	1	180.97	6.152	3.54	1.38	**0.01**
4	%_Hardwood3	3	183.178	8.36	1.17	5.3×10^{-1}	**0.03**
5	%_ Forestry3	3	185.053	10.235	-9.18	5.91	0.12
6	%_Resident	1	185.755	10.937	2.02×10^{-2}	1.33×10^{-2}	0.13
7	%_Occupation	1	187.508	12.69	3.23×10^{-2}	3.71×10^{-2}	0.38
8	%_BD_Higher	1	188.017	13.199	-9.39×10^{-3}	1.90×10^{-2}	0.62
9	%_Children_U6_B6_17	1	188.049	13.231	3.12×10^{-2}	6.60×10^{-2}	0.64
10	Pop_Density	1	188.212	13.394	5.62×10^{-4}	2.39×10^{-3}	0.81
11	%_ Tempfor	1	188.245	13.427	-1.99×10^{-3}	1.35×10^{-2}	0.88
12	%_Industry	1	188.266	13.448	2.77×10^{-3}	8.02×10^{-2}	0.97

CHAPTER 4

TREE SPECIES AND OVIPOSITION SITE SELECTION IN THIRTEEN-YEAR

PERIODICAL CICADAS WITHIN AN ANTHROPOGENIC FOREST LANDSCAPE[4]

Understanding the ecological and evolutionary consequences of dispersal is

important for predicting how species respond to changes in the physical environment due

to natural and anthropogenic activities such as habitat fragmentation, climate change, and

pollutants (Bowler & Benton, 2005). Dispersal, as defined as any movement of

individuals or offspring across space, has significant implications for genetic diversity at

local and global scales (Ronce, 2007). Particularly, a species' adaptive potential to

environmental changes is strongly influenced by dispersal and therefore has significant

effects on range, abundance and population dynamics (Bonte et al., 2012). Understanding

the factors that affect the dispersal ability of a species can provide insight into patterns of

extinction and speciation and potentially provide an early monitoring system for

assessing the quality of the environment for conservation efforts and policy making

(Parmesan, 2006; Santamaria & Mendez, 2012).

An important question regarding animal dispersal addresses how it varies with

ecological conditions. Specifically, insects have shown varying dispersal abilities under

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a range of ecological conditions, making the taxonomic group particularly sensitive to changes in habitat structure and abiotic cues (Robinet & Roques, 2010; Hill et al., 2011; Schowalter, 2012). For example, massive outbreaks and range shifts have been attributed to factors such as resource availability (Wilson & Richards, 2000), changes in temperature, photoperiod and humidity (Berg et al., 2006; Beuzelin et al., 2009) in addition to synthetic contaminants (Gate et al., 1995; Riesch et al., 2007; Scheirs et al., 2006). The resulting changes in behavior and movement can disrupt community dynamics, leaving a habitat predisposed to future disturbances (Schowalter, 2012).

Many insect species rely on tree hosts for mating, oviposition and feeding. Thus, changes in host represent immediate environmental challenges that affect early life cycle stages and subsequent adult fitness (Walsh 1864; Feder and Filchak 1999; Berlocher and Feder 2002; Malausa et al 2008). These challenges include the host's chemical defense (Fogleman 2001), food resource quality (Agrawal 2000) and exposure to novel bacteria and fungi (Janson et al., 2008). Specifically, variation in host trees may influence body size in insects, which is closely associated with fecundity, survival and longevity (Awmack & Leather, 2002) and has subsequent effects on development time (Iranipour et al 2010), attracting mates, oviposition schedule and immunocompetence (Murphy, 2007; Yang et al., 2008; Diamond & Kingsolver 2011).

Thus, changes in the tree host availability can have significant implications for the viability and abundance of vulnerable species at the landscape scale (Ulyshen et al., 2010). Human activity such as timber harvest practices dramatically change forest ecosystems with the removal of trees that result in increased habitat patchiness and reduced connectivity. Shifts in range and behavior have been documented in a variety of

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forest dwelling species including amphibian, bird, and small mammal species (Semlitsch, 2000; Sullivan et al., 2001; Millington et al., 2011). Additionally, Zetter et al. (2004) detected significant changes in ant species assemblages in response clear-cutting practices in deciduous forest sites with native populations decreasing while non-indigenous fire ant populations (*Solenopsis invicta* and *Pheidole spp*.) increased.

Insect species drawn to edges of habitat patches for mating and oviposition may be particularly sensitive to changes in forest patchiness and subsequent tree host availability. For instance, previous studies on periodical cicada (*Magicicada sp.*) behavior suggested dispersal was influenced by sunlight, temperature and disturbance history of the forest area (Williams & Simon, 1995; Rodenhouse et al., 1997; Yang, 2006). Furthermore, females tend to select trees for ovipositing eggs that are farther away from large chorusing aggregations (Williams & Simon, 1995) and on broader and longer branches (Mattingly & Flory, 2011), suggesting a selection pressure for decreased nymphal competition. Past accounts have suggested low dispersal from the emergence site and the distribution of the offspring generation to be similar to that of the parental generation (Karban, 1981; Oberdörster & Grant, 2006). However, recent studies have detected greater than expected dispersal ability and the influence of large scale landscape structure on cicada abundance suggests additional ecological factors may be driving movement across space (Logan et al., 2011; Beasley et al., 2012).

The aim of this study is to determine the degree to which tree host availability influences patterns of oviposition site selection in 13 year periodical cicada along the woodland edges of a working forest. We used a natural experimental design that incorporates maintained fields in the Long Cane Ranger District of the Sumter National

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Forest. Stand type and stand origin data derived from Sumter National Forest GIS data were used to randomly select four fields bordering mixed pine-hardwood forests. We controlled for sunlight exposure by selecting field edges with a western aspect because cicadas prefer to lay eggs on aspects with more light exposure (Yang, 2006). If cicadas are demonstrating a preference for a specific tree hosts, such as oak species, then we expect to see a clustering of oviposition scarring as the availability of that species decreases along a forest edge. If there is not a strong preference then we would expect a more diffuse rate of scarring along a forest edge. The results of this study will provide insight into how periodical cicadas respond to frequent changes in habitat structures due to large scale human disturbance.

2. Materials and Methods

2.1 Study Site

The Long Cane Ranger District of Sumter National Forest is currently composed of approximately 48.562 hectares located in the Piedmont physiogeographic province of South Carolina (Latitude: 33.8037, Longitude: -81.9308). Management practices began in 1936 for timber production, wildlife protection and recreation. The area is characterized by hardwood (*Quercus sp., Liquidambar styraciflua, Carpinus caroliniana, Ulmus alata*), pine (*Pinus taeda*) and mixed pine-hardwood forest stands.

2.2 Study Species

In spring of 2011, periodical cicadas of Brood XIX emerged throughout the southeast United States. Brood XIX consists of three species (*M. tredecassini, M. tredecim, M. tredecula*) that emerge synchronously every 13 years in the southeastern

United States. Males and females emerge from the ground close to their host tree and eclose into their adult form (Beasley et al., 2012). Adults live above ground for approximately 4-6 weeks. Both sexes are attracted to male chorus aggregations in the forest canopy. After mating, females disperse to nearby trees and repeatedly inject the ovipositor into the underside of a branch, leaving a characteristic scar with 20-30 eggs per egg nest (White et al., 1982; Williams & Simon, 1995) (Figure 1). Nymphs hatch from eggs after 6-8 weeks and drop to the ground where they feed on root xylem fluids for another 13 years.

2.3 Field Procedure

We used a random walk sampling protocol at each field edge that consisted of walking a randomly selected pace and setting up a ten meter transect where we sampled all tree species within the transect. For each tree, we identified species using a field guide (National Audubon Society, 1980) and measured the diameter breast height (DBH). We randomly removed three branches from each tree that fell within the range of 3-13 mm to search for oviposition scars and measured branch length and width (mm) (Clay et al., 2009). We measured oviposition scar length (mm) using digital calipers (VWR International, West Chester, PA) and nest counts as our measures of oviposition site preference with longer scar lengths and higher counts indicating higher preference (Mattingly & Flory, 2011).

2.4 Statistical Analysis

We categorized trees by species to account for the diversity of tree hosts along an edge (Table 1). We categorized the availability of the tree along an edge by calculating proportion of the ten meter transect the tree took up. Prior to analysis, we used the Kolmogorov-Smirnov test to check the distribution of data and square root-transformed data that was not normally distributed (oviposition scar length). We used a linear mixed model approach to test for differences in oviposition scar length and number of nests according to tree species applying PROC MIXED in SAS 9.3 (SAS Institute, Cary, NC, USA). We separately tested two response variables (scar length and nests) one at a time as a function of tree species (as a fixed factor) and availability and average branch diameter (as covariates). In addition, we included in the model the interaction between tree species and availability. We also included identity of the field site as a random effect to account for the effect of the sampling protocol on the outcome. We used the Wald test (z) as the test of significance for the random effect. This is equivalent to the F test used to test significance of our fixed effects (SAS Institute Inc., 2008).

We applied an Akaike information criterion (AIC) approach for model selection, sequentially removing the least significant terms, starting from the interactions. We removed a term from the model only if removal did not increase the AIC values by more than 2.00 (Burnham & Anderson, 1998).

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Results

3.1 Oviposition Scars

We first analyzed habitat preference as indexed by length of the oviposition scar. Tree species and the availability along an edge did not have a joint effect on the length of the oviposition scar. We did detect a significant main effect of tree species on oviposition scar length with conifer trees such as cedar (coefficient = -1.80×10^{-2} (S.E. = 1.31) t_{428} = -0.14, p = 0.89) and loblolly pine (coefficient = -1.66 (S.E. = 1.30) t_{401} = -1.27, p = 0.20) having shorter scars compared to other tree species. However, given the literature widely supports conifer trees being less preferred habitat sites for oviposition in cicada, we removed conifer trees from further analysis to determine if there was a preference for specific hardwood tree species. We did not detect a joint or independent effect from tree species after the removal of conifer trees from analysis (Table 2). Trees with larger average branch diameters had comparatively longer oviposition scars (Table 2).

3.2 Number of Nests

The effect of tree species on number of nests depended on its availability along the edge (Table 3). The number of nests in Hornbeam (*Carpinus sp.*) significantly increased as more of the species was available along the edge (coefficient = 15.85 (S.E. 6.47), t_{455} = 2.44, p = 0.01). Conversely, the number of nests significantly decreased in Red Oak (*Quercus rubra*) as more of the species was available on along the edge (coefficient = -3.92 (S.E. = 1.70), t_{76} = -2.31, p = 0.02). All other tree species interactions were non-significant (p > 0.11).

In addition, field site did not have a significant effect on oviposition scar length or number of nests ($z > 0.44$, $p > 0.27$), suggesting our sampling protocol did not affect the results of our study.

Discussion

In our study, we aimed to determine if 13-year periodical cicadas showed preference for specific tree species for oviposition after controlling for known physical characteristics within a working forest landscape. We expected highly preferred trees to have longer scars and more nests and the degree of scarring to depend on the trees availability along the forest edge. Overall, we detected the predicted preference for hardwood trees in comparison to conifer trees (White, 1982; Cook et al., 2001) but did not find a significant preference for hardwood tree species as indexed by oviposition scar length. Trees with larger branch diameters had comparatively longer scars, which is consistent with previous studies (Mattingly & Flory, 2011). When we measured habitat preference by nests, we found that the number of nests to vary amongst tree species depending on its availability along the forest edge. Some tree species such as Hornbeam had a higher number of nests as its availability increased along an edge while other tree species such as Red Oak had fewer nests as its availability increased along an edge, suggesting that cicadas do not appear to be constrained by seeking out a specific host and will disperse along a forest edge as more diverse trees become available. This suggests a more opportunistic strategy in habitat selection rather than a preference for a specific tree species.

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Tammaru et al. (1995) found similar results in the oviposition behavior of the Autumnal moth (*Epirrita autumnata)* and suggested that the physical characteristics of the tree host were more important than host quality or preference. For example, in addition to the diameter of available tree branches, the smoothness of the tree branch may influence oviposition preference. We found that tree species such as Winged Sumac (*Rhus copallina*) and Persimmon (*Diospyros virginiana*) had fewer nests compared to other hardwoods. Cook et al. (2001) also found fewer cicada oviposition scar damage on Smooth Sumac (*Rhus glabra*) compared to other hardwood trees.

The outcome of this study suggests that habitat connectivity is important for understanding cicada movement and abundance in an area and suggests that the long distance dispersal observed in some studies may be an indication of increased habitat fragmentation forcing individuals to disperse further to find suitable habitat. Conversely, it may also explain why some areas experience high population densities of cicadas. For instance, low dispersal and thus, indiscriminate oviposition preference may be in response to an energy tradeoff between egg production and flight performance for cicada females. Female cicadas demonstrate very little feeding behavior as adults and suggest a strong dependence on energy allocated during larval stages (Williams and Simon, 1995). Thus, increased fragmentation resulting in increased selection pressure on energy allocation towards dispersal ability and could significantly constrain a population over time. Future studies may want to compare number of nests laid by individuals in relation to dispersal from emergence source to better understand the impacts of increased dispersal due to habitat fragmentation.

In conclusion, ecosystems are undergoing rapid and intense changes due to increasing anthropogenic influence, global climate change and alterations in habitat structure resulting in changes in dispersal processes and long term consequences for global biodiversity (Santamaria & Mendez, 2012). Additionally, the incorporation of understanding animal responses to changes in habitat quality will better allow us to assess restoration efforts and improve harvesting practices to more effectively compensate ecological dynamics (Lindell, 2008). The next 13-year *Magicicada* emergence will occur in 2024. To better understand the consequence of changing ecological structure on cicada dispersal and subsequent abundance, future studies will want to use previous research to monitor changes in spatial distribution in relation to changing landscape structure (Beasley et al., 2012). Additionally, changes in plant community structure such as increased abundance in planted loblolly pine due to increased timber harvest activity, Winged Sumac or Persimmon can potentially have negative consequences on cicada populations over time.

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Table 1: Tree species encountered during this study.

Tree Species	Species Name
Winged Elm	*Ulmus alata*
Willow Oak	*Quercus phellos*
Cedar	*Juniperus virginiana*
Dogwood	*Cornus florida*
Post Oak	*Quercus stellata*
Persimmon	*Diospyros virginiana*
Loblolly Pine	*Pinus taeda*
Holly sp	*Ilex sp.*
Pumpkin Ash	*Fraxinus profunda*
Winged Summac	*Rhus copallinum*
Sweet Gum	*Liquidambar styraciflua*
Live Oak	*Quercus virginiana*
Red Oak	*Quercus rubra*
Hornbeam	*Carpinus caroliniana*
Water Oak	*Quercus nigra*
Privet	*Ligustrum vulgare*
Sparkleberry	*Vaccimium arboreum*
White Oak	*Quercus alba*
Honey Locust	*Gleditsia triacanthos*

Table 2: Results from linear mixed model for oviposition habitat preference as measured by oviposition scar length. Statistically significant relationships (p < 0.05) are marked in bold.

Final Model	Coefficient	S.E.	d.f.	F	P	AIC
Species*Availability			12, 153	1.72	0.07	3038.7
Species			12, 41	1.24	0.29	
Availability	1.80×10^{-1}	1.04	1, 709	1.1	0.29	
Diameter	3.34×10^{-1}	5.36×10^{-2}	1, 696	38.9	**< 0.01**	

Table 3: Results from linear mixed model for oviposition habitat preference as measured by number of nests. Statistically significant relationships (p < 0.05) are marked in bold.

Final Model	Coefficient	S.E.	d.f.	F	P	AIC
Species*Availability			12, 230	1.43	**< 0.01**	3331.8
Species			12,74.2	1.61	0.11	
Availability	4.76×10^{-1}	1.33	1, 713	0.48	0.49	
Diameter	2.40×10^{-1}	6.56×10^{-2}	1, 703	13.33	**< 0.01**	

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Figure 4.1. Periodical Cicada oviposition scars from spring 2011 emergence event, Long Cane Sumter National Forest, Edgefield, South Carolina
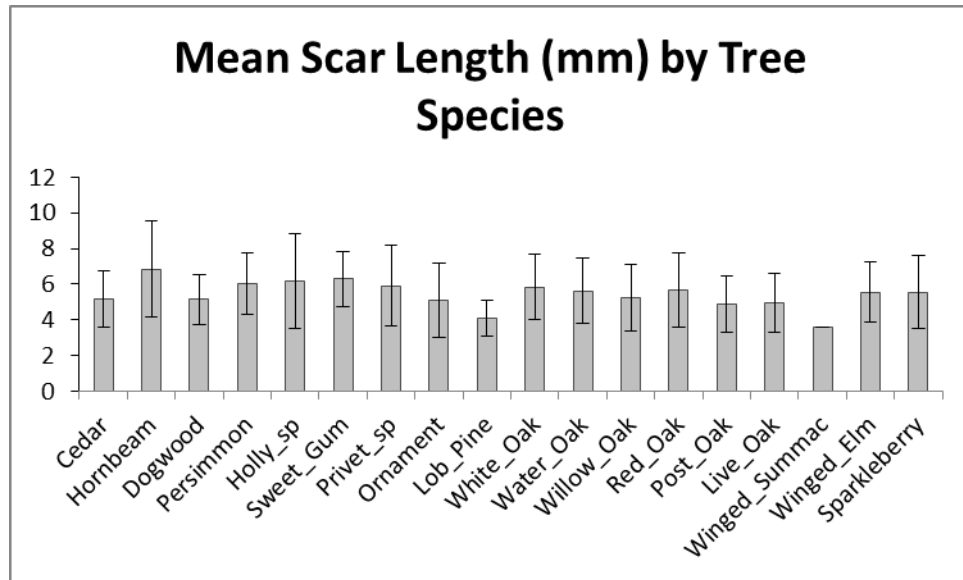
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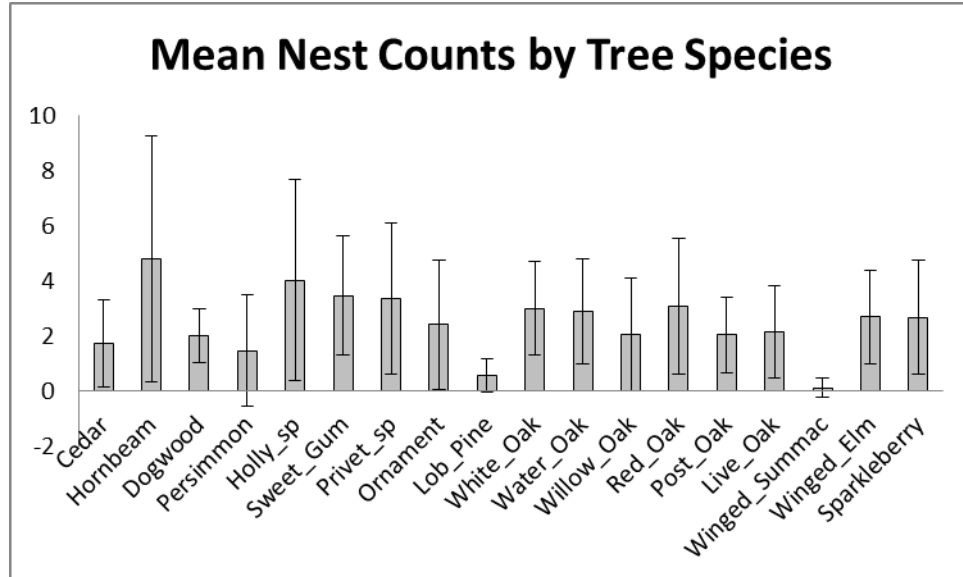Figure 4.2. Mean (± SD) scar length (mm) by tree species

Figure 4.3. Mean (± SD) nest count by tree species

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APPENDIX A – THE USE OF CITIZEN SCIENTISTS TO RECORD AND MAP 13-
YEAR PERIODICAL CICADAS (HEMIPTERA: CICADIDAE: MAGICICADA) IN
SOUTH CAROLINA[5]

Citizen science is the process in which citizens are involved in science as
researchers (Carr 2004). This can range from involvement in a collaborative effort from
academic and government agencies in the assessment of a common environmental
(community) concern to the management of common natural resources (Conrad &
Hilchey 2011). Traditionally, the use of citizen scientists involved the collection of data
on species detection and habitat. This is due to the difficulty of a single monitoring
agency in obtaining accurate data for proper population assessment (Conrad & Hilchey
2011). Increasing concern regarding the effect of climate change and land development
on biodiversity has raised interest in assessing evolutionary responses of plants and
animals, particularly for species that are absent in some years but abundant in others
(Heliovaara et al. 1994; Lepetz et al. 2009).

Brood XIX of periodical cicadas (Hemiptera: Cicadidae: *Magicicada*) consists of
3 species (*M. tredecassini, M. tredecim, M. tredecula*) that emerge synchronously every
13 years. Historically, Brood XIX has been recorded to span along the United States east
coast from Maryland to Georgia and in the Midwest from Iowa to Oklahoma (Simon
1988). Males and females emerge from the ground close to their host tree and eclose into

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their adult form. Adults are approximately 4 cm long with red eyes, orange-veined wings and black bodies (Fig. 1). They live above ground for approximately 4-6 wk. The males produce species-specific calls that attract both sexes in the forest canopy resulting in large aggregations in areas of high population densities. After mating, females disperse to nearby trees and lay eggs into slits cut into the branches. Nymphs hatch from eggs after 6-8 wk and drop to the ground where they feed on root xylem fluids for another 13 yr (White & Strehl 1978; Williams & Simon 1995).

In South Carolina, the 1985 emergence began around 21 Apr in Abbeville and Saluda counties. Cold weather delayed the emergence in 1998 resulting in fewer sightings. Based on anecdotal and general accounts, periodical cicadas were suspected to emerge in approximately 25 South Carolina counties (Gorsuch 1998). The anticipated 2011 Brood XIX emergence provided a unique opportunity to apply the use of citizen scientists in obtaining a more accurate assessment of the species range in South Carolina. The aim of the project was to use voluntary internet reports of sightings to examine spatial and temporal patterns of periodical cicada emergence and document citizen's impressions of the Brood XIX emergence.

Three South Carolina state institutions established internet sites for citizens to report sightings (Clemson University: http://clemsoncicada.wordpress.com/; University of South Carolina: http://cricket.biol.sc.edu/cicada/; and the South Carolina Forestry Commission: www.state.sc/forest). Local, public media that spanned into Georgia, South Carolina and North Carolina were used to inform citizen scientists of websites for reporting emergences. In addition, emails were sent out to professional groups such as County Extension Agents, Master Gardeners, the South Carolina Pest Control

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Association and the South Carolina Nurseryman's Association to inform them of the websites. Sightings were biologically validated with pictures, sound recordings, descriptions and in some cases by visiting locations and capturing individuals. Citizen scientists were asked to report the county, address, GPS coordinates and/or street intersections of emergence locations. Reports were categorized into fine or coarse scale depending on details available in the report. If not provided, decimal latitude and decimal longitude coordinates were determined using Google Earth software (Google Inc., Mountain View, California) and plotted onto a geographic information systems (GIS) map.

Citizen scientists' perception of the emergence event was categorized qualitatively via assessment of general comments. Responses were categorized as positive if comments included words such as "enjoyable", "amazing", "exciting", "neat" and "interesting". Negative comments included "terrible", "infestation", "irritating" or "nuisance". Responses were categorized as neutral if citizen scientists only gave basic information about the sighting with no or vague impressions about the emergence.

Reports of emergences occurred from early Apr to early Jun with the majority of sightings occurring from mid-Apr to mid-May. Overall, 191 location reports were received from 129 individuals from South Carolina, Georgia and North Carolina. In South Carolina, the emergence occurred primarily in the Piedmont region from 129 site locations and reported by 113 individuals (Fig. 2). Of those reporting, 90 reported on their experience or feelings about the emergence. From those comments, 30 (approximately 33%) perceived the experience as positive, 17 (approximately 19%)

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perceived the experience as negative and 43 (approximately 48%) did not comment on their perception of the experience.

The need for citizen science has increased due to various factors including the need for decision makers for timely information regarding the local environment, cuts in funding to monitoring programs and the desire for citizens to contribute to environmental protection/conservation (Whitelaw et al. 2003). Additionally, due to the rare and sporadic sightings of some species of interest, local citizen scientists are often best placed to monitor ecological patterns as they occur (Carr 2004). In 2011, periodical cicada emergences were reported in 19 South Carolina counties. Due to lack of exact location sightings from previous emergences (1998, 1985, 1972), we cannot determine if the current population suffered declines due to factors such as climate change, land development and timber harvests. However, the data and field experience obtained in this collaborative effort provides a significant benchmark for future studies on periodical cicada ecology and behavior in South Carolina. For example, the literature suggests that periodical cicadas rarely fly distances greater than 50 m from where they emerged (Karban 1981), however reports and field observations suggests a greater than anticipated dispersal ability. The cicada monitoring project also presented an opportunity for scientific public outreach. Citizen scientists expressed a range of positive accounts of the periodical cicada emergence from rescuing emerging adults from predation, photography, home-school education opportunities, to a willingness to provide additional monitoring information throughout the emergence event. Citizen scientists also actively engaged in scientific inquiry by presenting hypotheses regarding the cicada's behavior (i.e. attracted to light, attracted to large and/or oak trees) based on their observations and in some cases,

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pursued independent research via internet sources. Negative accounts such as expressing irritation about the noise level, concern about property damage and/or danger to pets and requesting information about insecticides indicate opportunities for promoting better understanding about cicada biology.

Recommendations for improving future cicada monitoring projects include: 1) coordinating interested South Carolina institutions and agencies in 2023 to create one common website with one supervisor, 2) implementing early outreach efforts to inform citizen scientists on what to expect from the upcoming emergence to reduce negative reactions and 3) include a short questionnaire to more accurately assess citizen scientists' perception of the emergence experience.

Figure A.1. Periodical cicada nymph from Brood XIX in South Carolina molting into the adult stage. Photos by Gerry Carner, Clemson University
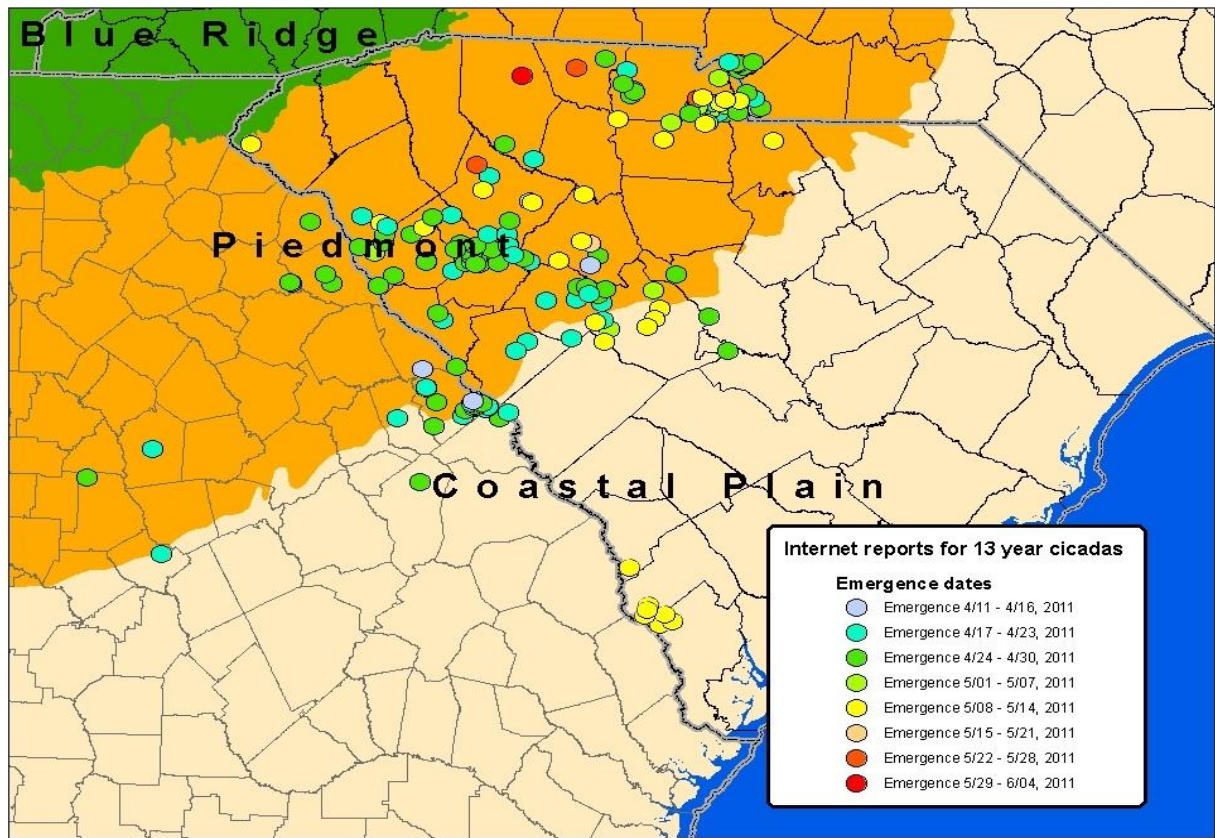
Figure A.2. Location and emergence dates of the 2011 13-year periodical cicada (Brood XIX) in South Carolina

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