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Population sizes and within-deme movement of *Trimerotropis saxatilis* (Acrididae), a grasshopper with a fragmented distribution

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Abstract Capture-mark-recapture studies were initiated in 1990 on four Missouri populations of the lichen grasshopper, *Trimerotropis saxatilis*. This grasshopper lives only on glade habitat, predominantly in the Ozark Mountains. Genetic data suggest that no gene flow occurs among *T. saxatilis* populations. Lichen grasshopper population size (both present and historical), and the likelihood of movement within and between glades, are the subjects of this study. Population sizes on all glades were found to be small (<280 individuals) and to vary from year to year. Inbreeding effective sizes were found to be much larger than census sizes. On one of the sites, Graham Cave Glade, population size was calculated for 5 years; in 3 of those years (1991, 1993 and 1994) our studies of this population also tested for movement of *T. saxatilis* individuals among different regions of the moderately subdivided glade. Maintenance of Graham Cave Glade (burning and clearing) was initiated after the 1991 capture-mark-recapture season. Comparisons of before- and after-burning intraglade movement probabilities did not show a significant difference. Grasshoppers more frequently remained in the part of the glade where they were previously captured, but were able to move about the moderately subdivided glade. The presence of a closed-canopy forest, rather than distance, appears to be an effective dispersal barrier.

Key words Population size · Inbreeding effective size · Capture-mark-recapture · Intrademic movement · Gene flow barriers

Introduction

The distribution of intraspecific genetic variation is affected by population structure parameters, including the degree of subdivision within a species, the amount of gene flow between populations, the size of those populations and the system of mating (Wright 1932). The effects of these parameters on patterns of genetic variability can be seen long after a change in population structure has occurred. For example, past bottleneck events (i.e., small population size) can be responsible for low levels of genetic variability seen in species of large population size (e.g., the northern elephant seal, Bonnell and Selander 1974). Fragmented populations may show levels of genetic variability that are indicative of their former widespread distribution, rather than their presently constrained migratory patterns. This happens if not enough time has passed since the fragmentation event for informative mutations to accumulate (e.g., the African elephant, Georgiadis et al. 1994). Patterns of genetic variability that suggest a particular population structure may, in fact, reflect historical rather than ongoing population-level processes if the species in question has undergone recent change in one or more of these parameters. Changes in population size and distribution are common in temperate species as a result of the significant climatic changes associated with the advance and retreat of Pleistocene glaciers, and more recently, the effects of human disruption of habitat. Untangling the contribution of historical from current population structure to levels of genetic variability is a non-trivial problem (Larson 1984; Larson et al. 1984; Slatkin 1987). When possible, it is best to empirically determine present-day population structure parameters to compare with genetic data.

One species whose population structure is expected to have changed over the last several thousand years is the lichen grasshopper, *Trimerotropis saxatilis*. This cryptic bandwinged grasshopper, whose distribution is primarily in the Ozark Mountains, is limited to glade habitat (McNeill 1901; Morse 1907; Otte 1984). Glades are relictual deserts and dry prairies: xeric rocky knobs or balds

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usually found on southfacing slopes. Glades are left over from the hypsithermal, a time 8,000 to 4,000 years before present (ybp) when the Ozark region experienced hot dry weather, and the desert/dry prairie expanded to its fullest extent (Webb 1981; Delcourt et al. 1986; COHMAP 1988). During this time *T. saxatilis* and other desert-adapted plants and animals arrived in the Midwest (Templeton et al. 1990; Sexton et al. 1992; Gerber 1994). The end of the hypsithermal (roughly 4,000 ybp) was geologically sudden (Chumbley et al. 1990), and the cooler, moister climate appears to have been more hospitable to forest growth (Nelson and Ladd 1980). As the Midwest was reforested, prairie and glade habitat was fragmented. Although under pressure from encroaching trees, prairies and glades were maintained for the last 4,000 years by periodic burning by Native Americans (Beilmann and Brenner 1951), resulting in a savanna grassland (Ladd 1991). Many plant and orthopteran species endemic to glades have adapted to a regimen of regular burning, requiring low-intensity fires for the following season's survival (Ladd 1991; Ballard 1992; Ballard and Greenlee 1994).

Increased rainfall in the last several hundred years (Beilmann and Brenner 1951) and/or settlement of the Midwestern plains has resulted in further glade fragmentation. Destruction of the prairie to create farms, and resettlement of Native Americans onto less desirable lands meant cessation of regular burning. Forests have rapidly invaded former prairie and glade habitat. Many glades recorded at the turn of the century by McNeill (1901) and Morse (1907) have been overgrown, and remaining glade habitat is considered endangered in the state of Missouri, where restoration efforts include cutting of juniper trees (a primary invader of rocky substrate) and regular burning. Today, glade habitat is patchily distributed throughout the Ozarks, and is inhabited by peripheral populations of such widespread xeric species from the southwestern United States as the collared lizard, *Crotaphytus collaris*. Like the collared lizard, lichen grasshoppers appear unable to move through the surrounding forest habitat, and are therefore "stranded" on glades, with no gene flow between populations (Gerber 1994).

In a species-wide survey of mitochondrial DNA (mtDNA) variation in *T. saxatilis*, ten individuals from each of 63 populations were examined via the polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs) (Gerber 1994; A.S. Gerber, unpublished work). Of those, 41 populations show high levels of variability (three or more mtDNA haplotypes), and only seven populations appear fixed for a single haplotype. These latter populations may have lost their genetic variability due to the effects of genetic drift on small population size: the random fixation and loss of haplotypes. Analyses of the genetic data indicate that populations are highly subdivided with no gene flow (Gerber 1994; A.S. Gerber, unpublished work).

In this paper we report the results of capture-mark-recapture experiments we did to determine sizes of four Missouri populations of *Trimerotropis saxatilis*, surveyed

over 5 years. Two of these populations (Graham Cave Glade and Sandy Ridge East) are among the 63 populations mentioned above. Graham Cave Glade has four haplotypes, Sandy Ridge East has two (Gerber 1994). As discussed below, these data indicate populations of sufficient size that extinction is unlikely and genetic drift a weak force. The parameter of true interest is N_e , the inbreeding effective size (Wright 1931). The vast majority of the population genetic literature, particularly that related to conservation biology, has emphasized that a population's actual size (as we estimate here) is in many cases greater than the inbreeding effective size (Gregorius 1991; Kimura 1983). However, there are conditions under which the inbreeding effective size can be larger than the census size (Templeton 1980), and these conditions are frequently encountered with endangered species (Templeton and Read 1994). A common situation that occurs in conservation biology is that of a declining population size (or an increasingly fragmented population). Under these conditions, the inbreeding effective size can be much larger than the census size, even by orders of magnitude (Templeton 1980; Templeton and Read 1994). If these grasshoppers have experienced increased fragmentation and/or decreased population sizes with the suppression of fire, it is possible that their current census sizes may be smaller than their inbreeding effective size. We wish to examine this possibility.

To begin to qualitatively understand the role that intrusive tree growth plays in fragmentation of glade habitat, and therefore its importance in subdividing lichen grasshopper populations, we also tested for movement within the fragmented glade, Graham Cave Glade, before and after removal of such growth. The two glades on Sandy Ridge (East and West) are separated by a narrow band of closed canopy forest. Comparisons between the ability of lichen grasshoppers to cross barriers at these two localities tell us something about the forces that have acted to fragment *T. saxatilis* populations in the absence of fire.

Methods

The following studies were conducted from 1990 to 1994. *T. saxatilis* is univoltine, so each year's individuals represent a new generation. Grasshoppers were captured with insect nets, marked, released and recaptured over a period of several days or weeks on each glade. Except as noted below, every effort was made to capture all *T. saxatilis* on a glade at each recapture visit. Two methods were used to mark captured grasshoppers; different colored acrylic paint dots, and pinpricks in specific bands on the wing. The former method was found to be more useful, and was used on all populations but one (see below). Marked animals were released in the same area where they were captured.

Study areas

Proffit Mountain, in Reynolds County, Missouri, has glades on exposed outcrops of Precambrian igneous rhyolite. The *T. saxatilis* population on one glade there (Proffit Mountain 3; 37°32'34"N, 90°48'35"W) was collected three times in a 3-week period in July and August 1990 and four times over a 2-day period in August

1993. In both years, animals from each collection event were marked with different colors of paint (e.g., on capture day 1, all animals caught were painted blue; on day 2 all animals were painted yellow).

Sandy Ridge, in Jefferson County, Missouri (38°17'5"N, 90°27'57"W), is a linear sandstone/dolomite glade separated by patches of juniper forest. Two populations (East and West, one on either side of a forest intrusion, separated by about 30 m of closed canopy) were collected four times over 5 days in August 1990, four times over 3 days in July 1993; and three times over 2 days in August 1994. In 1990 one of the populations was marked using paint dots, and one was marked using pinpricks in the forewing. In 1990, no attempt was made to collect all areas of the glade, so these data will be used only to estimate migration across the forest intrusion. In 1993 and 1994 extensive efforts were made to census the entire glade area. In 1993 and 1994 the two grasshopper populations were painted a different color on each collection date so that movement between the two sites could be detected, as well as population sizes determined (e.g., on capture day 1, all animals from Sandy Ridge East were painted green, those from Sandy Ridge West were painted yellow).

Graham Cave Glade, in Montgomery County, Missouri (Graham Cave 2, 38°54'11"N, 91°34'15"W), is a sandstone glade where the population size of lichen grasshoppers was estimated in all 5 years. This glade is surrounded by oak and juniper trees. In 1990, at the beginning of our study, it was fragmented by intrusive tree and grass growth. In September 1991 the glade was surveyed using a Sunto surveying compass and optical distance-measuring devices [made by Ranging Measuring Systems, East Rochester, New York, models 120 (2–30 m) and 620 (15–180 m), see Fig. 1]. We identified three large areas of exposed bedrock, the preferred habitat of lichen grasshoppers, so that the amount of movement between areas, as well as an estimate of the population size, could be determined. Area 1 was separated from the other areas by scrub and grasses with no exposed bedrock. Areas 2 and 3 were separated by a nearly continuous line of juniper trees growing in a region of only sparsely exposed bedrock. In February 1992 the Missouri Department of Natural Resources cut juniper trees and burned the glade. This maintenance reduced tree cover and rejoined some fragments, although it did not increase the area of exposed bedrock. The glade was burned again in February 1993 and February 1994. By August 1994, the last year of this study, the junipers between areas 2 and 3 no longer constituted a nearly continuous line.

In 1990 the Graham Cave Glade lichen grasshoppers were captured seven times over 15 days in August and September. Different colors of paint were used to mark animals captured at each visit (e.g., on capture day 1 all animals caught were painted blue). In 1991 the glade was visited four times in September. In that year, grasshoppers were *uniquely* painted, so that each individual could be identified in the future, and its movement tracked from one section of the glade to another. That area where an individual was first seen at each visit was recorded. In 1992 two collecting trips were made to Graham Cave Glade in September. In 1993 four trips were made over 5 days during August and September. In 1994 three trips were made over 4 days during August. In 1993 and 1994, individuals were again uniquely marked with paint, as in 1991.

Statistical analysis

Chao et al. (1992) developed a non-parametric method for estimating population size for capture-mark-recapture data when capture probabilities vary by time and individual. This method uses sample coverage estimates (the relative fraction of the total individual capture probabilities of captured animals) to measure completeness of the sample taken. Section 3 of Chao et al. (1992) describes how they use these sample coverage estimates to estimate population size, using three different sets of approximations ("models" in their paper). Their method is most useful when sample coverage is >50%. In this study all sample coverages were greater than 50% (see Fig. 2). We estimated population size and standard deviation of the estimators under all three models given in Chao et al.

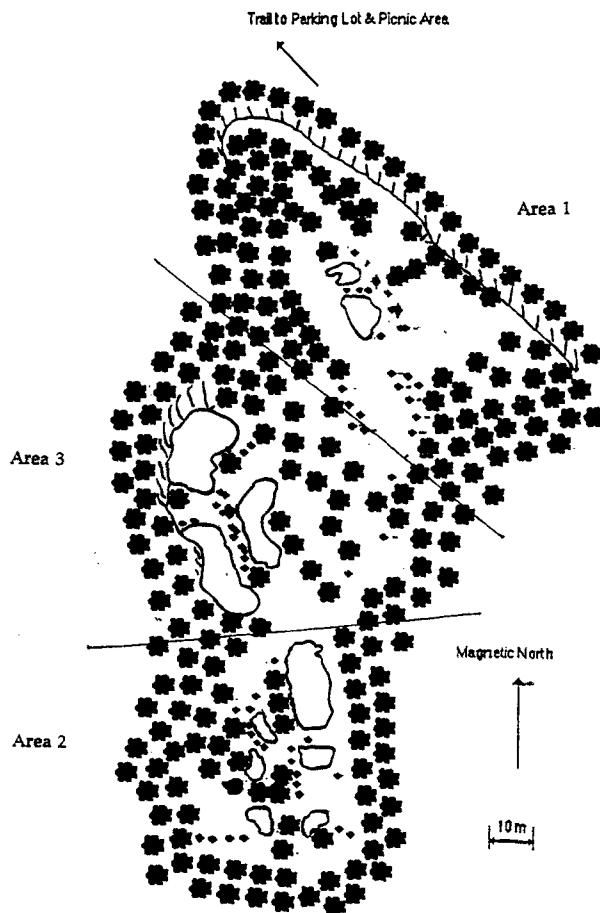


Fig. 1 1991 survey map of Graham Cave Glade. The *outlined areas* represent the rock faces where *Trimerotropis saxatilis* were caught. These were named *areas 1, 2 and 3*, and the *straight lines* superimposed over the map show how the glade was divided into these areas. Trees and bushes are represented by *black shapes* surrounding the glade. The *short lines* in areas 1 and 3 show the ridge edges. The burning and clearing of trees from this glade begun in 1992 did not change the area of the rock face, but removed the trees and shrubs from between the three areas

(1992). A program in Mathematica (Wolfram 1991) was written by A.R. Templeton to implement the models of Chao et al. (1992) and is available upon request from A.R. Templeton. For each case we chose as our final estimator that model resulting in the smallest estimated standard deviation, and with an estimated sample coverage ≤ 1 (estimates > 1 indicate that the model assumptions are inappropriate for that data set).

The method of Chao et al. (1992) was used to estimate population sizes for all data except the 1992 Graham Cave Glade data. Because Graham Cave Glade grasshoppers were captured only twice in 1992, the algorithm is not useful. Two other methods of analysis were used to estimate the 1992 Graham Cave Glade population size, and compare it to the other capture-mark-recapture studies. First, a program written by A.R. Templeton for the Hewlett Packard calculator HP-41, which uses the Lincoln Index hypergeometric sampling assumption (Johnson and Kotz 1969, p. 147), was used to estimate population size. To compare the 1992 Lincoln Index-analysed Graham Cave Glade data to the rest of the data, we analysed only the first 2 days of recapture data for all the 1990, 1991, 1993 and 1994 surveys using this method (see Table 1). Second, all days of recapture data were subject to the Mean Petersen estimate method (Table 1) (Seber 1973, p. 138).

We used maximum likelihood to estimate the migration parameter m , the probability of *movement per day* from one section of

Graham Cave Glade to another, for the 1991, 1993 and 1994 data (see Tables 2–4). To determine the estimator \hat{m} , the recapture data for each animal was rewritten as $(1-m)$ for each day the animal remained on the same section of glade and m for each day the animal moved to another part of the glade (see Tables 2–4). We used the parsimonious assumption that if an animal was found in a particular area of the glade on two successive captures then it had remained there, and had not moved away and returned in the intervening time. If the grasshopper was found on a new part of the glade, we assumed that it moved only once during the time between captures. This assumption is more likely to have been violated in 1991, when four collection trips were distributed over 15 days, than in 1993, when four trips were distributed over 5 days, or in 1994 when three trips were distributed over 4 days.

When capture dates were separated by more than 1 day, the actual number of days between capture was taken into account when assigning a probability to the observed dispersal behavior of an individual (p_i). For example, in 1991, one animal (see Table 2, 4th row) was found in area 1 three times, between 7 September and 14 September. The assumption of parsimonious movement gives this animal a value of $(1-m)^7$, because over the course of 7 days it was found in the same part of the glade. However, another animal was captured twice, in area 2 on 7 September and in area 1 on 12 September (see Table 2, 6th row). These dates are 5 days apart; therefore, this animal has a p_i value of $m(1-m)^4$. This value conveys the parsimonious assumption that although the animal moved once (represented by m), it did not move elsewhere for the other 4 days [represented by $(1-m)^4$].

The likelihood equation is proportional to the product of all the p_i observations:

$$L \propto \prod_{i=1}^{45} p_i = m^9(1-m)^{173}$$

To determine the maximum value of \hat{m} , the migration parameter estimator, we took the first derivative of the natural logarithm of the likelihood equation, set it equal to zero, and solved the equation for m . To determine the variance of \hat{m} , we took the second derivative of the natural logarithm of the likelihood equation. For example, the observed numbers of dispersal and non-dispersal events for 1991 are 9 and 173, respectively (see Table 2). To obtain their expected values, note that $9+173=182$ days of total dispersal opportunities were observed. The expected number of dispersal events is $182m$ and the expected number of non-dispersal events is $182(1-m)$. Therefore, the expected value of the second derivative of the natural log likelihood is

$$-\frac{182}{m} - \frac{182}{1-m} = -182 \left[\frac{1}{m(1-m)} \right]$$

The negative reciprocal of this value, using \hat{m} instead of m , is the estimated variance for \hat{m} .

Results

The population size estimates and sample coverages from the Chao et al. (1992) algorithm are given in Fig. 2. The population size estimates and standard deviations derived from the Lincoln Index scores and the Mean Petersen estimates are given in Table 1. The data from which these estimates are made are given in Table 5.

The glade at Graham Cave is roughly 7000 m² (see Fig. 1 for a map of the glade made in 1991, before cutting and burning). Intraglade movement of animals from the 1991 survey is shown in Table 2. The migration parameter estimator, \hat{m} , is 0.049, with standard deviation of 0.017. This is the probability that an animal on Graham Cave Glade would move to another section of the glade in one day in 1991. The intraglade movement of animals from the 1993 survey is shown in Table 3. The migration parameter estimator, \hat{m} , is 0.045, with a stan-

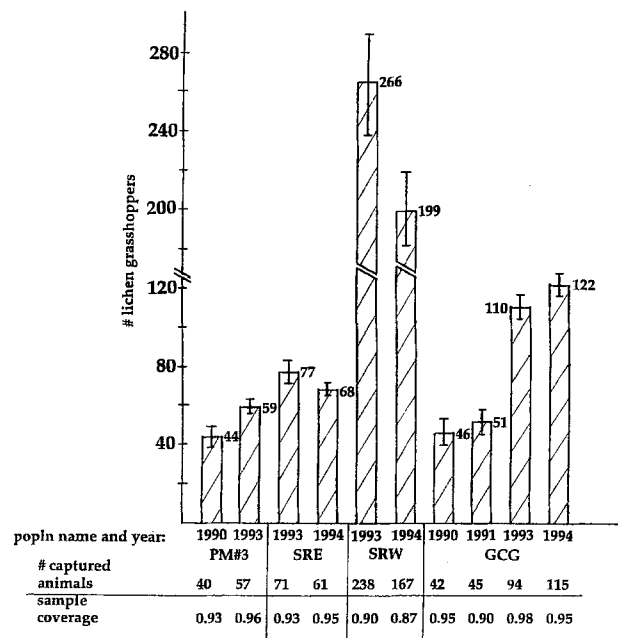


Fig. 2 Population size estimates and 95% confidence limits for the Chao et al. (1992) analyses. Below the x-axis the actual number of grasshoppers caught and the Chao et al. (1992) sample coverages are given for each population and year of the study

Table 1 Size estimates of all populations using the Lincoln Index (LIE) and Mean Petersen (MPE) estimates. The LIE are based on *only the first 2 days* of capture data for each glade. *Number of captured animals* is the number of animals captured in the first 2 days of collection, with the *total number* (all days) of captured animals in parentheses. The MPE were made using data from *all days* of capture. Standard errors for LIE and MPE are given in parentheses

Glade and year	Number of captured animals	Lincoln Index estimates (LIE) and SEs	Mean Petersen estimates (MPE) and SEs
Proffit Mtn #3 1990	38 (40)	40.7 (7.7)	39.8 (0.7)
Proffit Mtn #3 1993	49 (57)	54.7 (10.3)	57.4 (1.5)
Sandy Ridge E 1993	67 (71)	84.0 (16.2)	75.3 (2.4)
Sandy Ridge E 1994	52 (61)	55.0 (8.8)	57.8 (0.9)
Sandy Ridge W 1993	177 (238)	277.6 (43.1)	275.0 (10.3)
Sandy Ridge W 1994	149 (167)	170.8 (20.5)	233.7 (23.0)
Graham Cave 1990	27 (42)	32.9 (9.2)	41.7 (1.7)
Graham Cave 1991	41 (45)	52.0 (13.9)	47.0 (1.9)
Graham Cave 1992	74 (74)	96.3 (18.5)	95.0 (8.49)
Graham Cave 1993	91 (94)	94.4 (11.3)	93.7 (0.5)
Graham Cave 1994	114 (115)	118.3 (13.1)	115.9 (1.0)

Table 2 Dates and places of capture for grasshoppers on Graham Cave Glade in 1991. Refer to Fig. 1 for area locations. Animals with two glade areas listed for a single date were captured twice in one day; p_i values were generated to calculate the maximum value of the migration parameter \hat{m} . Animals captured only once have no p_i value, and are not included in this table. Number of observed migration events (m)=9; number of non-migration ($1-m$) events =173. See text for further discussion

Number of animals	7 Sept	9 Sept	12 Sept	14 Sept	p_i
1	Area 1	1			$(1-m)^2$
2	1	1		1	$(1-m)^7$
1	1	1	1		$(1-m)^5$
1	1		1	1	$(1-m)^7$
4	2		2		$(1-m)^5$
1	2		1		$m(1-m)^4$
2	2			2	$(1-m)^7$
2	2		2	2	$(1-m)^7$
2	2	2			$(1-m)^2$
1	2	2		2	$(1-m)^7$
2	2	2	2		$(1-m)^5$
1	2	3			$m(1-m)$
1	2	3	3	2	$m^2(1-m)^5$
2	3		2	2	$m(1-m)^6$
1	3	3/2	2	2	$m(1-m)^6$
1	2		2	3	$m(1-m)^6$
1	3		3	3	$(1-m)^7$
1	3		3		$(1-m)^5$
1	3	3	3/1	1	$m(1-m)^6$
2		2	2	2	$(1-m)^5$
1		2		2	$(1-m)^5$
1		3	3		$(1-m)^3$
2			2	2	$(1-m)^2$
1			3	3	$(1-m)^2$

dard deviation of 0.012. For 1994 the intraglade movement of animals is shown in Table 4. The \hat{m} is 0.071 with standard deviation of 0.014. Pooling the data from 1991, 1993 and 1994, \hat{m} is 0.057 with a standard deviation of 0.008.

The log-likelihood ratio test of the null hypothesis of homogeneity of the migration ratio in 1991, 1993 and 1994 is 2.222, which should be distributed as χ^2 with 2 df under the null hypothesis. There are no statistically significant differences in intraglade dispersal rates on Graham Cave Glade in 1991, 1993 and 1994.

No movement was detected between the two glades on Sandy Ridge in 1990, 1993 or 1994.

Discussion

To characterize a species' population structure by surveying genetic variability is a method that is rapidly becoming more useful, as theoretical tools gain in statistical strength and sophistication. However, no theoretical construct can eliminate the lag time between a change in population structure and the genetic evidence of that change. Whenever possible, studies of population structure examined by geographic distribution of genetic variability (known as intraspecific phylogeography, Avise et al. 1987) should be augmented by ecological data (Palumbi et al. 1991).

Table 3 Dates and places of capture for grasshoppers on Graham Cave Glade in 1993. Number of observed migration events (m)=13; number of non-migration ($1-m$) events = 275

Number of animals	28 Aug	29 Aug	30 Aug	1 Sept	p_i
15	Area 1	1	1	1	$(1-m)^4$
1			1	1	$(1-m)^2$
3	1	1		1	$(1-m)^4$
2		3		1	$m(1-m)^2$
16	2	2	2	2	$(1-m)^4$
2	2	2		2	$(1-m)^4$
1	2		2	2	$(1-m)^4$
1	3	2	2	2	$m(1-m)^3$
1	1	1	3	2	$m^2(1-m)^2$
2		2	2	2	$(1-m)^3$
1	3	2		2	$m(1-m)^3$
1	3	3		2	$m(1-m)^3$
1	3			3	$(1-m)^4$
4		3	3	3	$(1-m)^3$
14	3	3	3	3	$(1-m)^4$
2	2	2	3	3	$m(1-m)^3$
1	1	3	3	3	$m(1-m)^3$
1	2		2	3	$m(1-m)^3$
1	2		2		$(1-m)^2$
1		2	2		$1-m$
1	3	3	3		$(1-m)^2$
1	1		1		$(1-m)^2$
3		1	1		$1-m$
4	1	1			$1-m$
1	3	1			m
3	3	3			$1-m$
3	2	2			$1-m$
1	3	2			m

Table 4 Dates and places of capture for grasshoppers on Graham Cave Glade in 1994. Number of observed migration events (m)=24; number of non-migration ($1-m$) events = 313

Number of animals	9 Aug	11 Aug	12 Aug	p_i
25	Area 1	1	1	$(1-m)^3$
1	1		1	$(1-m)^3$
3		1	1	$1-m$
28	2	2	2	$(1-m)^3$
2	3	2	2	$m(1-m)^2$
5	2		2	$(1-m)^3$
5		2	2	$(1-m)$
1	3		2	$m(1-m)^2$
15	3	3	3	$(1-m)^3$
3	3		3	$(1-m)^3$
21	2	2	3	$m(1-m)^2$
3	1	1		$(1-m)^2$
5	2	2		$(1-m)^2$
5	3	3		$(1-m)^2$

Population sizes

There is general agreement between all three methods of population size estimation (see Fig. 2 and Table 1). We found population sizes of four lichen grasshopper demes to be small (44–266 individuals on glades using the Chao et al. (1992) estimates; 33–278 individuals using the Lincoln Index and Mean Petersen estimates. All but

two of these estimated population sizes are less than 125 individuals).

These census estimates are too small to support the high levels of genetic variability we see. To demonstrate this, we examine the Graham Cave Glade data. The four mtDNA haplotypes found in that population define a total of 27 different restriction sites, three of which are polymorphic. Using Eq. 26 in Ewens (1983; note that Eq. 26 was inadvertently not labeled) with $k=3$, $m=27$, $j=4$ and $n=10$, the estimator of the nucleotide heterozygosity is 0.014 in this population. Under the neutral theory, the expected heterozygosity should be $4N_e\mu$. For mtDNA, which is haploid and maternally inherited, the expected heterozygosity (in terms of N_e for the entire population, assuming a 50:50 sex ratio) is $N_e\mu$. We can estimate the inbreeding effective size from genetic data for Graham Cave Glade as $0.014/\mu$, where μ is the per nucleotide/year mutation rate. In order to get N_e of the same order of magnitude as our post-burn census size (200), μ would have to be of the order of 10^{-5} . Recent estimates of μ for insect mtDNA are of the order of 10^{-8} (Brower 1994). Therefore, the inbreeding effective size is several orders of magnitude larger than the census size. This discrepancy can be reconciled by a reasonable scenario: prior to fire suppression by settlers, the glade populations were not only bigger, but there was gene flow among them (Gerber 1994). At that time, the effective size did not correspond just to a local glade population, but could effectively encompass many different glade populations. With fire suppression the glades were fragmented (Gerber 1994) and now the demographic unit of a glade has also become the genetic unit of a deme. This resulted in a drastic reduction of local breeding size, but because inbreeding effective sizes are reduced at a lower rate than census sizes in a declining population, it would lead to the current situation in which the inbreeding effective size is much larger than the census size. For example, suppose that the presettlement Graham Cave Glade population had many more haplotypes than seen today because of genetic connections with other populations (remnants of these glades exist nearby in the Loutre River valley). Suppose, for example, it had 15 haplotypes. Fragmentation causes an immediate demographic isolation but drift works more slowly, causing a slow reduction in the number of haplotypes from 15 (or whatever it was) to the current 4, which is still more than should exist in a constant-sized glade population of 100–200 individuals. If fragmentation continues, we would expect further loss of haplotypes on this glade, and the inbreeding effective size and the census size would gradually converge. However, Graham Cave Glade has not arrived at this point of convergence, and the current levels of genetic variation in the population still reflect its recent past history rather than its current demographics.

Our population size and genetic data show that it is not correct to assume (as is commonly done) that effective sizes are always smaller than census sizes. Moreover, declining populations can still retain high levels of

genetic variation despite small current size. Small populations should not be automatically excluded from conservation programs, as there can still be much genetic diversity left to conserve.

Active maintenance such as that carried out on Graham Cave Glade since February 1992 will become necessary to prevent the overgrowth of remaining glades by forest and the continued diminution of glade endemic populations. Small populations such as Proffit Mountain 3 are particularly susceptible to random extinction events.

It is interesting to note that the population size estimates increased on Graham Cave Glade following the commencement of glade maintenance. Although within our study this is only a single data point, the suggestion that clearing and burning the glade resulted in larger population size for *T. saxatilis* is in keeping with a study by the Missouri Nature Conservancy (Ballard and Greenlee 1994) that found that habitat types in Missouri (including glades) that received annual burning consistently showed greater orthopteran abundance and diversity than similar habitats that were burned less frequently or only grazed.

Grasshopper movement within and between glades

In the 1991, 1993 and 1994 capture-mark-recapture studies at Graham Cave Glade, we marked each grasshopper uniquely so that particular individuals could be identified when found on subsequent recapture visits. Thus we were able to follow the movement of all individuals over time across the glade. Graham Cave Glade was of particular interest to us because it was already subject to intrusive growth by juniper trees, the primary invader of glade habitat. Lichen grasshoppers can fly distances in excess of the total length of Graham Cave Glade, but all lines of evidence examined (including genetic data and the existence of apparently appropriate glades with no *T. saxatilis* present) suggest that lichen grasshoppers in the Ozarks do not migrate from glade to glade through forest, and that each population is an independently evolving genetic entity. By examining the ability of lichen grasshoppers to move around a glade that was in the process of fragmentation, we hoped to understand the nature of barriers to gene flow in this species.

In the 1991 study at Graham Cave Glade, grasshoppers were more often found on the part of the glade where they had been previously captured (see Fig. 1, Table 2). Glade area 1 is the most distinct from other parts of the glade, being separated from the nearest region, area 3, by about 20 m of scrubby trees and grass (not closed canopy), and is the only region of the glade not visible from the rest of the glade, due to the slope of the hillside. Only three animals from areas 2 and 3 were found to have migrated to area 1 during the 1991 study. No animals from area 1 were later captured on any other area of the glade (this is not unexpected since there are fewer animals on area 1 than the rest of the glade). In

Table 5 Capture-mark-recapture data used to estimate population sizes. The Sandy Ridge 1990 data are not included here because

the collection method differed from that employed at all other sites, and population sizes for Sandy Ridge 1990 were not estimated

Glade	Capture dates	Number of new & recaptured animals/visit	Glade	Capture dates	Number of new & recaptured animals/visit
Proffit Mountain 3 1990 (total animals captured=40)	25 July	31 new	Sandy Ridge W 1993 (total=238)	29 July am	132 new
	31 July	7 new		29 July pm	45 new
		28 recap		31 July am	40 recap
	10 August	2 new			56 new
	27 recap	31 July pm		83 recap	
					5 new
Proffit Mountain 3 1993 (total=57)	1 August	39 new		63 recap	
	2 August	10 new	Sandy Ridge W 1994 (total=167)	29 July am	123 new
		28 recap		29 July pm	26 new
	3 August	7 new			18 recap
	26 recap		31 July	18 new	
Graham Cave Glade 1990 (total=42)	22 August	21 new		104 recap	
	23 August	6 new			
		12 recap			
	28 August	7 new			
		8 recap			
	29 August	5 new			
		17 recap			
	30 August	1 new			
		21 recap			
		2 new			
Graham Cave 1991 (total=45)	2 September	20 recap			
	9 September	0 new			
		16 recap			
	7 September	37 new			
	9 September	4 new			
		13 recap			
Graham Cave 1992 (total=74)	12 September	3 new			
		20 recap			
	14 September	1 new			
		21 recap			
Graham Cave 1993 (total=94)	4 September	47 new			
	5 September	27 new			
	26 recap				
Graham Cave 1994 (total=115)	28 August	77 new			
	29 August	14 new			
		70 recap			
	30 August	3 new			
	1 September	73 recap			
Graham Cave 1994 (total=115)		0 new			
		69 recap			
	9 August	105 new			
Sandy Ridge E 1993 (total=71)	11 August	9 new			
		83 recap			
	12 August	1 new			
		90 recap			
Sandy Ridge E 1994 (total=61)	29 July am	47 new			
	29 July pm	20 new			
		26 recap			
	31 July am	3 new			
	31 July pm	47 recap			
Sandy Ridge E 1994 (total=61)		1 new			
		39 recap			
	3 August am	44 new			
	3 August pm	8 new			
	39 recap				
	9 new				
	44 recap				

1993 two animals from area 3 dispersed into area 1, and two animals from area 1 were found later in area 3. Dispersal between areas 2 and 3 were more common in both years. In 1994 all movements were between areas 2 and 3.

Beginning in 1992, after the first year of within-glade movement data, Graham Cave Glade was cleared and burned every February. We did not anticipate this maintenance by the Missouri Department of Natural Resources, but it allowed us to test for differences between the likelihood that grasshoppers would move to different parts of the glade before and after the intrusive tree growth was removed. It is important to note that this maintenance did not increase the size of the habitat (exposed bedrock), but cleared trees from between sections of the glade, and prevented further destruction of the rock face. Because there is no evidence for heterogeneity between the 1991, 1993 and 1994 studies of intraglade movement, there is no detectable effect on intraglade movement due to fire treatment over the short term.

Lichen grasshoppers can fly a distance much longer than the 30 m separating the two sections of Sandy Ridge. However, in three years of study, no migrants were found between the two sections of Sandy Ridge. From these observations, and those at Graham Cave Glade, we conclude that the closed canopy and/or thick understory of mature forest intrusions, rather than distance, is the barrier to gene flow for these insects.

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