Reviewer Name	Chapter	Page	Line #	Comment
Hill	ex. summary	2	23	give scientific name for golden violet here at first mention
Hill	ex. summary	3	8	population
Hill	2	11	10&11	"created" should not be used, please change the sentence to: "A recent phylogenetic analysis confirmed the monophyletic (descended from a common ancestor) nature for the genus Speyeria (de Moya et al. 2017, p. 640)
Hill	2	11	14	delete evolutionary
Hill	2	11	18	italics for Speyeria
Hill	2	11	22	At about this point in the paragraph it makes sense to cite Thompson et al 2019 and discuss confirmation of the 16 species
Hill	2	11	32	separate instead of standalone; consider using 2018a and 2018b?
Hill	2	11	34	"to separate Bay Area Speyeria species, recovered each species, including S. callippe,"
Hill	2	12	15	Edit line to say "...butterflies show more mixing with Comstock's silverspot butterfly (S. callippe comstocki)."
Hill	2	12	15	Comstock's formal scientific name should be given at first mention
Hill	2	12	26	I suggest changing text to read: "that resemebled callippe, as well as comstocki and callippeXcomstocki intercross butterflies" Those names (cal-com and com-cal) are based on the closeness of phenotype and they may not even be hybrids, just variation.
Hill	2	12	24-27	This is also true for San Bruno Mtn.
Hill	2	13	17	I would cite the Hill et al 2018 separate "standalone" paper here b/c it analyzed S. z. sonomensis
Hill	2	13	18&19	remove 1 & 2
Hill	2	13	28	"and could not be included in the genetic study"
Hill	2	14	25	falls - s is missing
Hill	2	15	17-19	I think it is important here to mentiond the populations in the Diablo range can strongly resemble callippe callippe but show much more mixing with callippe comstocki - this is where some of the redundancy (increased genetic representation) could come from
Hill	3	16	9&10	remove 1 & 2
Hill	3	16	8	citation?
Hill	3	17	1	Give scientific name for golden violet
Hill	3	17	1	In the figure "After first instar diapause, larvae go through five more instars." There are six total instars, based on callippe comstocki and other Speyeria) and this has been misinterpreted in the literature.
Hill	3	17	figure lege	It is in an odd place in my version in Word

Hill	3		17	table 2	larva might get a light green box for feeding in March as well
Hill	3		19	4	edit "(cold blooded)" to be "(have a variable body temperature)"
Hill	3		19	26-27	Males eat less than females so they pupate sooner and are smaller - see Hill, Rush and Mayberry 2018. "Larval Food Limitation in a Speyeria Butterfly (Nymphalidae): How Many Butterflies Can Be Supported? Insects.
Hill	3		19	31-39	Callippe silverspot do not have reproductive diapause. They oviposit in May-June-July right away. The coronis in the Bay Area do not lay eggs until September and it is very clearly different. Edit line 31 to say "Callippe silverspot butterflies being ovipositing soon after mating,..." and delete the last sentence of the paragraph, or exchange with "Callippe silverspot show no indication of undergoing reproductive diapause". You can cite me as personal communication if needed. If someone does have evidence then it should be cited but I think the pattern is clear.
Hill	3		20	15	Cite the Zaman et al paper for S. adiaste here as well.
Hill	3		20	16-18	Cite the Zaman et al paper for S. adiaste here as well.
Hill	3	20-21		38,39 and	Is it worth mentioning that for callippe silverspot it is not only winter conditions, but dry hot summer conditions that may cause mortality as well?
Hill	3		21	5&6	I doubt there are some callippe doing 5 and some doing 6, and it is all 6 instars and Arnold's 5 means 5 in adition to the first, diapausing instar. We were very careful watching for molts and head capsules and never observed 5 instars and I don't think this is a result of captive rearing (I doubt Arnold could count instars in the field).
Hill	3		21	14-15	Cite the Zaman et al paper for S. adiaste here as well.
Hill	3		21	31-32	But fog is likely the reason for the melanic phenotype, which is a main reason why the species is recognized as distinct.
Hill	3		21	36	I would add along trees at the grassland border (not just riparian trees)
Hill	3		23	7	I would give the scientific name of the host plant here
Hill	3		25	1,2,3	This is also generally true for Speyeria
Hill	3		25	13	It is probably worth adding text here about our work on S. adiaste and leaf consumption, see citation above (row 26).
Hill	3		25	34-37	Another variable is how many leaves and how much leaf area, not just number of plants, see Hill, Rush and Mayberry 2018
Hill	3		26	2&3	citation?

Hill	3	26	16-20		In addition to topographical diversity, having diverse nectar plants: Monardella, Buckeye and thistes, ensures nectar is available throughout the flight season. This is especilly importan in years of early butterfly emergence when nectar may not be yet available, or years of late butterfly emergence when nectar may be declining already. See Zaman et al 2014.
Hill	3	26	26-27		If citing work on S. idalia then it is even more relevant to cite Zaman et al 2015 on S. adiaste which is much more similar to S. callippe. Zaman, Tenney, Rush and Hill. Journal of Insect Conservation(2015) 19:753-763
Hill	3	27	1,2,3		Here again, citing Zaman et al 2015 is also very relevant.
Hill	3	29	figure 7		Connectivity is affected by nectar plants (as indicated in observations above traveling 1.6km to get to Buckeye) and also to hostplants since females likely search for and find high host densities.
Hill	3	30	17-18		But wouldn't restoring a population such as in Joaquin Miller help in resiliency (and redundancy)?
Hill	4	31		10	3 seems out of place <--This is in hidden text...
Hill	4	33	2and 3		figure caption should indicate more about colors of shapes, shape meaning, color of arrows, etc. What is + and what is -?
Hill	4	34	line2		RDM not RSD
Hill	4	34	23-29		Some of the thistles, including star thistle, and mustards may also be adult nectar sources.
Hill	4	37		19	larvae instead of larva
Hill	4	39		10	habitat not habit
Hill	4	43		18	additional natives to mention would be Cobweb Thistle (C. occidentale) and Mule ear (Wyethia). Wyethia is common at Sears pt but dries up early, so could be important in early emergence years.
Hill	4	44	3to5		It is not clear which of the sites in table 7 are "populations" and which are not. There are 6 extant sites in table 7 but only 4 extant populations in this paragraph.
Hill	4	45	figs 10 and		Colors for transects should be the same in both graphs.
Hill	4	47	2, 20,		Lake Herman Blvd.?
Hill	4	49		21	oviposition
Hill	4	49	30-31		Please clarify, you mean "to have provided quantitative examples of sufficient numbers"? What are "quantitative examples", samples?
Hill	4	51	Table 8 foc		Bartolome or Bartalome?
Hill	4	51	Table 8		What are units for 0.36? The "point-" from line intercept sampling should be removed if this is my data. I used line intercept sampling estimating the linear distance of Viola pedunculata cover intersecting a transect (not just at points along a line).

Hill		4	51	Table 8	sufficient in Moderate is misspelled
Hill		4	51	Table 8	Any way to get more specific about "sufficient numbers"? At least one buckeye tree per square mile since that is what data say is possible? And some equivalent nectar volume of Mondardella. This column should be more quantitative if at all possible. How can it be repeated again with a different set of people using this document as a guide?
Hill		4	52	2and3	Table 6 is incorrect, it should be Table 8, right? And table 7 should be 9.
Hill		4	53	37	tend to be?
Hill		4	54	17	Sears Park should be changes to Sears Point
Hill		4	54	31	delete s on populations
Hill		5	55	7	six poplations are mentioned here but chapter 4 was using population for the Cordelia Hills group of sites. Language should be consistent across the entire document.
Hill		5	57	14	larvae instead of larva
Hill		5	57	28	adult females (no s on adult)
Hill		5	57	30	egg, larval, and pupal life stage.
Hill		5	58	19	abundance not abundances
Hill		5	58	20	"...in how climate change will affect local habitats and influence the species."
Hill		5	59	1	"Given this "business as usual" scenario,"
Hill		5	59	4	population (no s)
Hill		5	59	30	management should only increase in scenario 2, otherwise they overlap too much and the projections are too similar (p64 lines 30&31 say increases in management only)
Hill		5	60	10	20 years? Shouldn't it be 30 years?
Hill		5	60	28-30	edit this sentence - "condition" used too many times
Hill		5	61	5&6	Conclusions do not match the table 11 and Figure 16 - neight has any population in High overall condition
Hill		6	64	29	Says two here, above said three (on line 5 p61, previous comment)
Hill		6	65	12	population in two of the three (missing in)
Hill		6	66	Table 12	San Bruno is High but was not high in Table 11
Hill	references		67	3	missing italics and p in Lepdoptera
Hill	references	67+		all	check italics on plants and animals in all references; genus is italics and caps, species is italics lower case
Hill	references		69	20	Edwards paper - check caps, should not have species epithet capitalized
Hill	references		69	40	quercetorum should not be capitalized
Hill	references		71	5	silverspot, not two words

insects

MDPI

Article

Larval Food Limitation in a *Speyeria* Butterfly (Nymphalidae): How Many Butterflies Can Be Supported?

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Abstract: For herbivorous insects the importance of larval food plants is obvious, yet the role of host abundance and density in conservation are relatively understudied. Populations of *Speyeria* butterflies across North America have declined and *Speyeria adiaste* is an imperiled species endemic to the southern California Coast Ranges. In this paper, we study the link between the food plant *Viola purpurea quercetorum* and abundance of its herbivore *Speyeria adiaste clemencei* to better understand the butterfly's decline and aid in restoration of this and other *Speyeria* species. To assess the degree to which the larval food plant limits adult abundance of *S. a. clemencei* in 2013, we compared adult population counts to population size predicted from a Monte Carlo simulation using data for number of *V. pur. quercetorum* plants, number of leaves per plant, and leaf area per plant, with lab estimates of leaf area consumed to reach pupal stage on the non-native host *V. papilionacea*. Results indicated an average estimate of 765 pupae (median = 478), with 77% of the distribution being <1000 pupae. However, this was heavily dependent on plant distribution, and accounting for the number of transect segments with sufficient host to support a pupa predicted 371 pupae. The adult population empirical estimate was 227 individuals (95% CI is 146 to 392), which lies near the first quartile of the simulated distribution. These results indicate that the amount of host *available* to larvae was more closely linked to adult abundance than the amount of host *present*, especially when considering assumptions of the analyses. The data also indicate that robust populations require host density well in excess of what is eaten by larvae, in combination with appropriate spacing, to mitigate factors such as competition, starvation from leaving host patches, or unrelated to food plant, such as mortality from drought, predators, parasites, or disease.

Keywords: *Viola*; *Viola purpurea*; *Speyeria adiaste*; bottom–up; top–down; insect conservation

1. Introduction

Interactions among plants, herbivores and parasitoids/predators have received considerable attention in the ecological literature. Insect herbivores have played a prominent role in these studies to answer questions ranging from "why is the world green?" to "what is the relative role of bottom–up and top–down effects in controlling herbivore abundance?" to "what factors modulate resource limitation and predation in a system?" [1–5]. These classic ecological questions have been applied to conservation biology less often, yet they clearly relate to issues of population decline, restoration, and management of plants and herbivores [6–9]. For example, are species declining because of bottom–up resource limitation, or changes to top–down effects of predators or parasites, or an interaction between the two?

Studies of butterflies across the globe have documented marked decreases in populations [10–17]. A powerful example of this in North America is seen in the decline of populations of *Speyeria* butterflies along with their native *Viola* larval host plants [18]. *Speyeria* populations across North America have been affected, from the relatively large sized eastern North America species *Speyeria idalia* [8], and *Speyeria diana* [19,20], with their once large geographic distributions, to *S. nokomis* in the southwestern U.S. [18], to smaller sized species with many described geographic subspecies in western North America such as *S. hydaspe* [18], *S. zerene* [18,21], *S. egleis* [22], *S. adiaste* [23,24] and *S. callippe* [25]. Each of these species has reportedly declined in abundance or number of populations. Some of the taxa of western species have received federal listing as endangered (e.g., *S. zerene behrensii*, *S. zerene myrtleae* and *S. callippe callippe*), or threatened (e.g., *S. zerene hippolyta*), but the rest have not.

Reasons for decline of *Speyeria* butterflies center mainly on human habitat alteration and associated effects on their *Viola* larval food plants. *Speyeria* and *Viola* are very sensitive species, and human disturbances have been implicated in altering habitats to be less suitable for *Viola* food plants by cutting and thinning forests, overgrazing or water diversion ([18], RIH personal observation), or destroying habitats altogether via development, agriculture, and natural resource extraction such as mining ([18], RIH personal observation). Alteration of habitats resulting from fire suppression or prescribed fire, or overgrowth of non-native plants such as European grasses in California, have also been discussed [8,22,26]. Fire and grazing may have positive or negative effects in different systems depending on their intensity. For example, *S. egleis egleis* have been documented to colonize new areas after wild fire, with declines associated with succession and overgrowth afterward [22], and *S. idalia* larvae appear capable of surviving low to moderate surface fires [27]. For *S. diana*, prescribed fire appears to help with adult nectar sources [28], but repeated fire may harm *Viola* populations [8]. Overgrazing is detrimental to *Viola* [18], but the correct amount of grazing can be beneficial for managing grasslands by slowing or stopping succession and reducing non-native grasses that overgrow native plants such as *Viola* spp. In the California Coast Ranges many grazed grasslands show the most robust *S. callippe* and *S. coronis* populations (RIH personal observation). Climate change and drought are also implicated in declines of *Speyeria* populations [20,22,29] and presumably their *Viola* hosts.

Speyeria populations clearly need adequate *Viola* populations, but what adequate is, and many other aspects of *Speyeria* larval ecology have only recently been investigated or are not well understood. For example, Kelly and Debinski [8] demonstrated that larger populations of *S. idalia* were significantly correlated with larger habitat areas, and by extension access to more *Viola* food plants. However, adult *S. idalia* population size was not significantly correlated with *Viola* host density in their analysis, leaving unresolved the question of whether the food plant was limiting [8]. Data on biomass consumption of *Speyeria* larvae are still lacking, and as suggested by Kelly and Debinski [8], data on the biomass required by a larva, and an entire population, as well as how food limitation relates to fecundity, would be of conservation value. Other recent work has demonstrated that *Speyeria zerene* larvae did not locate their food plants well and only find them when they are very close (~3 cm) [30], and that host density is very important in modeling larval survival in this species [31]. Thus although it is clear that large amounts of dense *Viola* host will provide the strongest populations, the link between larval host abundance and adult abundance has rarely been investigated [8], and few data exist on field estimates of host abundance and density, leaving open the question of how much larval food plant is required for a given population size, and what the threshold abundance is to support a population [8]. Therefore, identifying the amount of *Viola* necessary to sustain a given *Speyeria* larval population until adult form is important for better understanding *Speyeria* larval ecology.

Here we focus on a particular species of conservation concern in southern California, *Speyeria adiaste* (W.H. Edwards 1864), to continue investigations of its autecology and answer to what degree the food plant is limiting. *S. adiaste* is endemic to the southern California Coast Ranges and Transverse ranges [32,33] and is currently without threatened or endangered status. Within *S. adiaste* are three described subspecies, which from north to south are *S. a. adiaste*, *S. a. clemencei*, and *S. a. atossa*. *Speyeria adiaste atossa* (Santa Barbara, Ventura, Los Angeles, and Kern Cos.) is considered

extinct because it has not been seen since 1960 [22], making *S. adiaste* the only *Speyeria* species to have a described subspecies go extinct (but see [34] for discussion of *S. zerene*). Of the remaining two subspecies, *Speyeria adiaste clemencei* (Monterey to San Louis Obispo Co.) is considered the most abundant, although the number of widely spaced remaining populations, and their sizes, remains unknown (but see [24]). *Speyeria adiaste adiaste* (San Mateo Co., Santa Cruz Co. and Santa Clara Co.) has become increasingly rare and suffered population declines. *Speyeria adiaste* has been petitioned for endangered status because of declining populations, but was rejected based on a lack of available information concerning the species. Recent work on *S. a. clemencei* documented population sizes of several hundred individuals on Chews Ridge at best, followed by marked declines during the study period [24]. This potential for drastic changes in population size coupled with the seemingly widely spaced populations of *S. adiaste* suggests this species exists as a metapopulation and will require adequate populations of *Viola* hosts to colonize and maintain viable subpopulations. However it is unknown what these *Viola* populations should look like. Thus, goals of this project are to provide baseline data on *Viola* food plant population abundance and density, and explore the link between food plant and *Speyeria adiaste* abundance. This information can elucidate how many adult butterflies a specific habitat should theoretically support and can be an important component guiding how *Speyeria* and *Viola* habitats should be managed and restored.

2. Materials and Methods

2.1. Study Site

Field portions of this study took place on Chews Ridge in Monterey Co., California (36.31336°; −121.57323°, 1500 m elevation) in 2013. This locality has a robust, but declining population of *S. adiaste clemencei* [23,24], and fieldwork was conducted in the same 243,206.5 m^2 area as in Zaman, Tenney, Rush and Hill ([24]; see Figure 1). The study area contains slopes of mixed oak-pine woodland having areas of relatively open yellow-pine and oak woods alternating with moist to dry meadows.

2.2. Abundance of Adult Butterflies and Food Plant

The larval food plant of *S. adiaste clemencei* at Chews Ridge is *Viola purpurea* Kellogg subsp. *quercetorum* (M.S. Baker & J.C. Clausen) R.J. Little [23]. We conducted transect counts to estimate the amount of available *Viola purpurea quercetorum* leaf area on Chews Ridge. On each transect the number of individual plants was counted, and these data were combined with data on the number of leaves per plant, the leaf area, and the total area of the study site.

Parallel belt transects (n = 135) were made every 16 m and extended from the lower study area boundary to the ridge crest. In total, 12,442 m of linear distance was sampled, with an average transect length of 92.16 m. A compass was used to orient each transect to 40° relative to true North to ensure transects were parallel. Each transect was broken into segments of 30 m maximum length (n = 475 segments overall, n = 371 of 30 m), and GPS data were recorded at the start and end of each segment. Approximately 270 person hours over 9 days of fieldwork were completed. *Viola pur. quercetorum* within one meter of each side of the transect line were counted (Figure 1A), giving a maximum sampling area of 60 m^2 per 30 m segment, and a total transect area of 24,883 m^2 sampled across the study site.

Viola pur. quercetorum individuals were relatively easy to distinguish, even when clumped together allowing us to tally the total number of individual violets. However, we also recorded data on cover by measuring the length along the longest dimension and width perpendicular to that, for each violet or patch of violets (Figure 1B). To obtain data for number of leaves per plant, we counted the number of leaves per plant on the first five to 20 plants in haphazardly chosen segments among 28 transects. In total 241 plants were sampled for leaf number per plant. Given that sample size was low for this variable based on data collection in 2013 (n = 85), we returned to collect additional data in 2014. There was no difference in mean leaf number per plant between years (equal variance t_{239} = 0.11, p = 0.91,

test with unequal variance assumption was qualitatively the same). To calculate leaf area, images were taken of up to 10 leaves per plant/patch, with each leaf placed under a metric printed grid (1 mm × 1 mm) placed on a white background (Figure 1C). Leaves on each plant were selected based on compass direction, beginning at due north, and continuing in a clockwise direction until up to 10 leaves were sampled. In total 349 leaves were sampled for leaf area.

The size of the *S. a. clemencei* population on Chews Ridge was estimated in a related paper using mark-release recapture methods in June and July 2013 (see [24] for details) and so we use those estimates here. The adults marked in June–July 2013 would have fed on the violets available during the April–May 2013 transect counts.

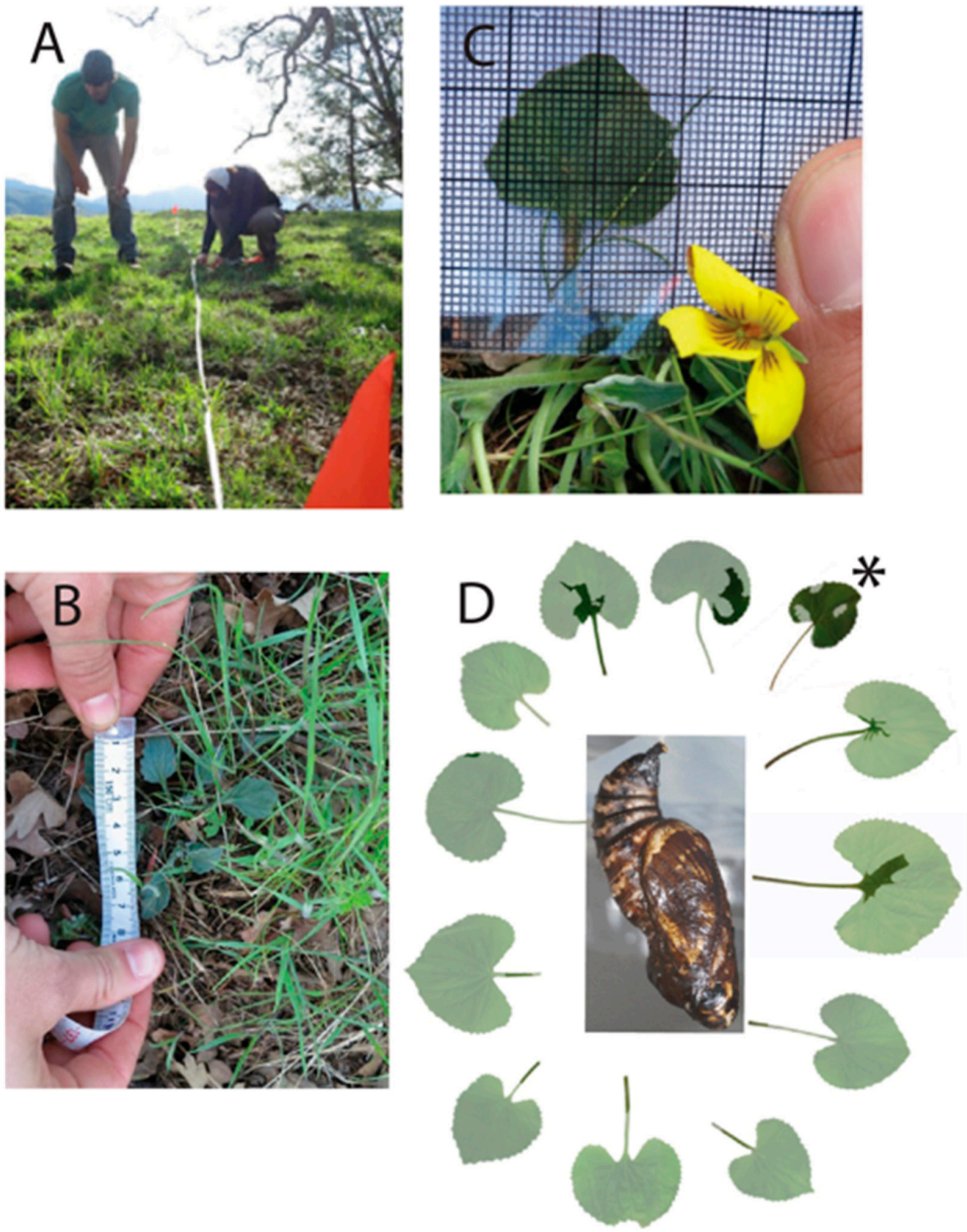

Figure 1. Estimating area of *Viola purpurea quercetorum* and area of consumed *V. papilionacea*. (**A**) Abundance transect on Chews Ridge. (**B**) Assessing coverage and number of leaves per plant. (**C**) Quantifying leaf area per leaf. (**D**) Consumption of rearing host *Viola papilionacea*, pale leaf shows plant before consumption and dark overlay shows remaining leaf after consumption. Asterisk indicates first leaf consumed, with subsequent leaves arranged clockwise.

2.3. Leaf Area Consumption

Speyeria adiaste clemencei were reared in the lab to obtain data on the amount of food plant required for a larva to reach the adult stage. A female *Speyeria a. clemencei* from Chews Ridge was placed within one day of field collection into a brown grocery bag with dried leaves of *Viola pedunculata* Torr. & A. Gray and *V. papilionacea* Pursh. Larvae were retrieved from the bags and put into wood blocks for diapause at 4 °C and later reared in plastic cups with cut leaves of *Viola papilionacea* (purchased from Tennessee Wholesale Nursery). See Zaman, Tenney, Brunell, Chen and Hill [23] for further details. Digital photos were taken of each leaf before and after being offered to a feeding larva, with the difference in area representing leaf area consumed. Spirit levels were used to ensure images were taken with the leaf and camera in the same plane, and the leaf was gently overlaid with a transparency sheet printed with a metric grid (1 mm^2 cells). Cells in the grid with more than half of the area containing leaf were counted. To estimate the amount of leaf area required to become a viable adult, the area consumed was summed across all leaves from first instar to pupation (Figure 1D). We recorded sex and measured forewing length for resulting adults. Forewing length was also measured for field collected males and females. We used an equal variance *t*-test to test the null hypothesis that male and female leaf area consumption was the same ($n = 4$ for each sex, test with unequal variance assumption was qualitatively the same). We used a two-way Analysis of Variance (ANOVA) with interaction to test for differences in forewing length (an estimate of size), between sexes and between lab reared vs. field collected individuals. In total, 10 larvae from the same female were reared on leaves from potted *Viola papilionacea*. This lab host was used because it is a readily available surrogate for wild California *Viola pur. quercetorum*, and no *V. pur. quercetorum* were available. In addition, the morphology of *V. papilionacea* is conducive to image analysis and measurement, *S. adiaste* larval survival was good, and it is more similar in water content to *V. purpurea* than store bought pansies, which can also serve as rearing food plants. Leaf area is an appropriate proxy for amount of food because it correlates strongly with mass [35] and is amenable to quantification before and after larval feeding without as large of an artifact from desiccation, as would be the case with mass.

2.4. Model Fitting and Simulation Analysis

To model the distribution of number of leaves per plant, leaf area per leaf, and leaf area consumed we fit the following distributions to each data set using maximum likelihood and the fitdistr() function in R: normal, log-normal, truncated normal, gamma, and Weibull. All but the truncated normal distribution are available as options in R, so we wrote our own code for this distribution (Appendix A). We selected the distribution with the lowest Akaike Information Criterion (AIC) [36] as the best model for each data set and plotted the fitted curves for each distribution to the data to assess the fit. Model fitting results are provided in Appendix B.

Given the large number of segments that lacked *Viola purpurea* individuals, the plant per area data set had many zero values requiring a different model fitting approach. For the plant per area dataset we fit an exponential distribution using maximum likelihood and compared this result to a model that was a mixture between a point mass at zero and an exponential distribution with weights estimated from the proportion of zero values in the data set. A likelihood ratio test was used to test for a significant difference between these nested models.

A Monte Carlo simulation estimating the number of pupae Chews Ridge could potentially support was performed using 10,000 iterations. In each iteration new values were obtained in the following way to model stochastic variation. Data for number of leaves per plant, leaf area per leaf, and leaf area consumed per pupa were obtained by drawing at random from their fitted distributions. Data on number of *V. purpurea* plants per m^2 was obtained by generating a distribution of 1000 data points based on the fitted mixture model and calculating the resulting mean. This reduced the amount of variation in density in each iteration but was necessary to avoid draws of zero violets. The estimated number of supported pupae in each iteration was calculated with the following formulae:

$$\text{Leaf Area Present} = \text{Area Sampled} \times V.\ purpurea/\text{m}^2 \times \#\text{Leaves Per Plant} \times \text{Leaf Area Per Leaf}, \quad (1)$$

$$\#\text{Pupae} = \text{Leaf Area Present} \div \text{Leaf Area Consumed}, \quad (2)$$

In order to model the effect of available food plant density on the variance in number of potential pupae, an additional simulation analysis was conducted using violet densities ranging from 0.01 to 0.1 plants per m^2 in 0.001 intervals. This range was chosen to encompass 71% of the empirical data. All modeling and statistics were done using R 3.4.0 [37].

3. Results

3.1. Viola Purpurea Quercetorum Abundance, Density and Amount of Leaf Present on Chews Ridge

In total, the transect counts found 1258 *Viola purpurea quercetorum* individuals. The *Viola pur. quercetorum* individuals were not uniformly distributed across the sample area (Figure 2). Instead, they were clumped, and most often found in the more moist woodland and woodland margins in small openings, from relatively flat to more sloped areas, rather than in the more open meadows. Based on the total transect area sampled (24,883 m^2) a rough calculation of *V. pur. quercetorum* density was 0.051 per m^2. However, this does not take into account variation between transects (Figure 2), which had a maximum density of 0.65 *Viola* per m^2 and minimum of 0.0 per m^2. Therefore a better approach was to estimate the density on each transect and average, which gave an average *Viola* density of 0.037 per m^2. The mixture model with zero point mass and exponential distribution was a significantly better fit ($\chi^2 = 573$, $p < 0.00001$) to the *V. pur. quercetorum* per m^2 data than the exponential distribution. The fit of the mixture model to the data is shown in Figure 3A.

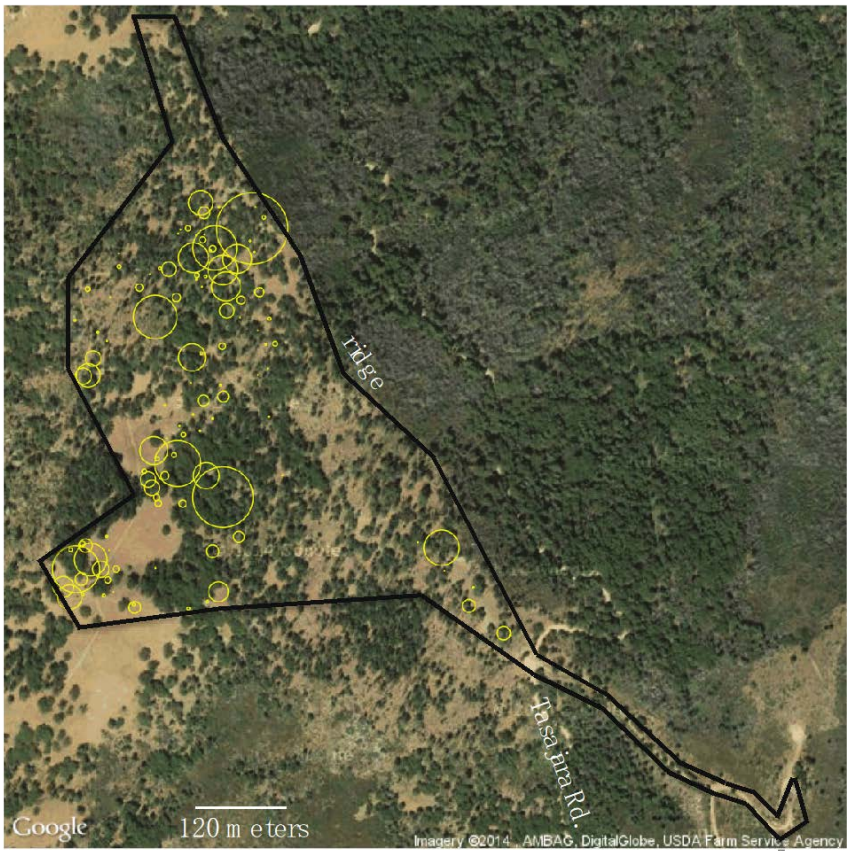

Figure 2. Distribution of *Viola purpurea quercetorum* in the study area. Yellow circles indicate number of *Viola pur. quercetorum* found in each 30 m transect segment. Transects ran diagonally from left to right approximately perpendicular to the ridge.

The average number of leaves per *V. pur. quercetorum* plant was 10.3, with a median of 8.5 leaves and standard deviation of 7.9 leaves (Figure 3B). The best fit to the leaf per plant data set was the log-normal distribution (AIC = 1561.5, see Appendix B: Table A1) with parameters meanlog equal to 2.05 and sdlog equal to 0.792.

The average leaf area per leaf of *V. pur. quercetorum* was 198.6 mm^2 with median 181.0 mm^2 and standard deviation 111.0 mm^2 (Figure 3C). The best fit to the leaf area per leaf data was the gamma distribution (AIC = 4219.5, see Appendix B: Table A2) with shape parameter equal to 3.00 and rate parameter equal to 0.0151.

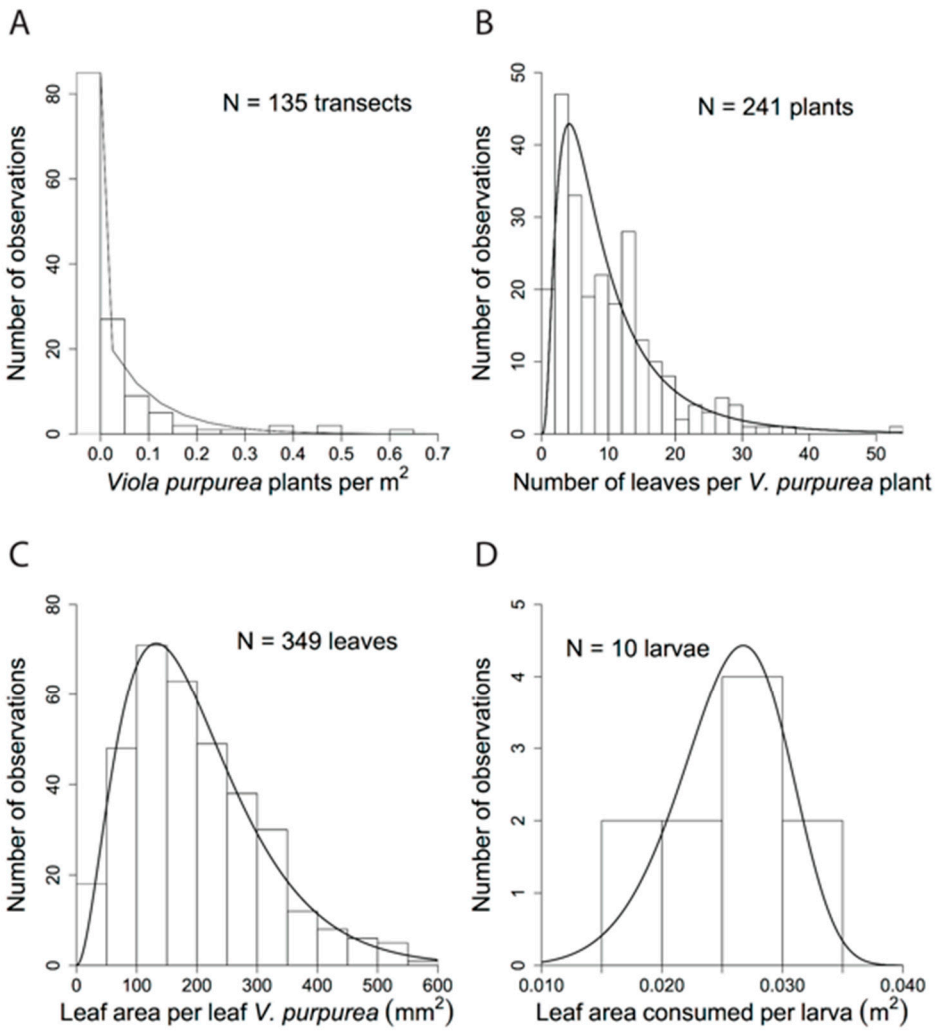

Figure 3. Data histograms and fitted distributions. (**A**) Number of *V. pur. quercetorum* plants per area. (**B**) Number of *V. pur. quercetorum* leaves per plant. (**C**) Variation in leaf area among *V. pur. quercetorum* leaves. (**D**) Leaf area of *V. papilionacea* consumed by *S. adiaste clemencei* larvae.

3.2. Food Plant Consumption in Lab

All 10 *Speyeria adiaste* reared in the lab pupated to provide data on leaf area consumed to pupation. Two individuals died during the pupal stage and did not eclose; however the leaf area consumed by these individuals was well within the range of the others, and the values suggested they were female. Mean leaf area consumed among all 10 larvae was 253.9 cm^2 (median = 277.9, s.d. = 53.2 cm^2) (Figure 3D), and ranged from 163.3 cm^2 to 310.3 cm^2. Males and females differed strongly in the leaf area consumed (t_6 = 5.5, p = 0.0015) with females consuming significantly more (mean = 290.6 cm^2, s.d. = 15.8 cm^2, n = 4) than males (mean = 196.7 cm^2, s.d. = 30.3 cm^2, n = 4).

The two-way ANOVA testing for effects of lab rearing and sex on forewing length of individuals was significant ($F_{3,13} = 10.0$, $p = 0.001$). Both sex ($F_{1,13} = 16.3$, $p = 0.0014$) and lab rearing ($F_{1,13} = 13.4$, $p = 0.0029$) had significant effects on forewing length. The interaction of sex and lab rearing was not significant ($F_{1,13} = 0.27$, $p = 0.61$), indicating that forewing length differences were consistent, with males and lab reared individuals being smaller. For wild females average forewing length = 31.56 mm ($n = 3$), versus an average of 28.75 mm ($n = 4$) for lab reared females. For wild males average forewing length = 28.91 mm ($n = 6$), versus an average of 26.71 mm ($n = 4$) in the lab.

The best fit for the leaf area consumption data used to draw from in the simulation analysis was the Weibull distribution (AIC = −74.6; see Appendix B: Table A3), with shape parameter equal to 6.53 and scale parameter equal to 0.027 (Figure 3D).

3.3. Simulation and Comparison of Estimated Number Pupae to Adult Population Size

Using the total area of the study site and data from the fitted distributions of *Viola pur. quercetorum* density, number of leaves per plant, leaf area per leaf, and leaf area consumed, resulted in an asymmetric distribution (Figure 4A) with the average number of potential pupae on Chews Ridge being 765. The median number of pupae supported was 478, with 25% of the distribution being below 237 and 95% of the distribution being below 2403 pupae. In comparison the 2013 MRR estimate [24] was 227 butterflies (95% CI 146 to 392). The MRR estimate is at 23.7% of the simulated distribution, with lower and upper 95% CI at 12.9% and 42.3% respectively. The simulation varying density showed a strong linear relationship of increased number of pupae with increasing density ($r^2 = 0.99$, $p < 0.00001$, $y = 12{,}759.7x - 0.103$, Figure 4B). As density increases, the variation in predicted number of pupae widens (Figure 4B) as a result of multiplying larger values of density by the other variables.

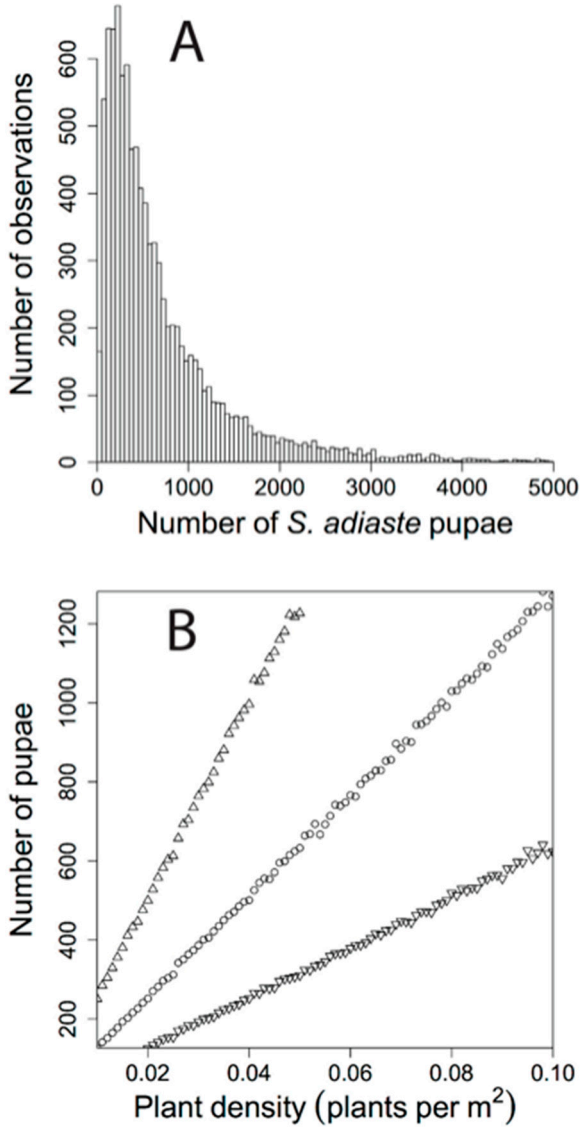

Figure 4. Number of pupae predicted based on simulations drawing from fitted data distributions. (**A**) Frequency distribution based on simulated results using variation in mean density. (**B**) Number of pupae resulting from simulations that vary density across natural values. Circles are median, upper triangles are 75th percentile and down triangles are 25th percentile.

4. Discussion

The question of whether *S. adiaste* is limited by larval resources ultimately involves asking how many adults there *should* be in a given site. This paper presents the first dataset we are aware of quantifying the number of butterflies supported by the biomass of a *Viola* population. Our studies indicate there were 227 adult butterflies in 2013 and although the amount of plant *present* appears adequate to support this number, the amount of host *available* to larvae was much more limited because of plant distribution and associated larval movement. At face value the *Viola* abundance and biomass in 2013 predicted more pupae in 50% of simulation runs, so why were there "only" 227 butterflies in 2013? Herbivores do not generally eat all their host so should we expect all the host to be used? We discuss these questions along with several assumptions of our analyses below, both with respect to *S. adiaste*, and also to *Speyeria* in general.

Our results describing variation in plant distribution, abundance, density and leaf area present for its herbivore *Speyeria adiaste clemencei*, enabled us to predict how many individuals this site could

sustain. The resulting asymmetric distribution of number of *S. adiaste* pupae strongly overlapped with the estimate and confidence interval for adult population size (Figure 4A), with the adult population size at approximately the 25th percentile of the distribution. However, given the asymmetric shape of the distribution of pupae, the 50th percentile was not far from the upper 95% CI of adult population size (392 adults vs. 478 pupae). Varying density had a strong effect on predicted number of pupae (Figure 4), with a positive linear relationship, similar to the results of Bierzychudek and Warner [31]. In our simulations it appears that only when relatively high values of plants per m^2, number of leaves and leaf area are combined with relatively low values of leaf area consumed do the results deviate strongly from the observed number of adults (see Figure 4A,B). For example if the density were twice our observed value the median number of pupae predicted was 944 (Figure 4B). Thus our analysis predicts that a few hundred, and certainly less than a thousand *S. adiaste* individuals, could be supported by the *Viola* biomass present in this study, but we should not expect a *Viola* population such as this to support more than a thousand, and definitely not thousands, of adult *S. adiaste*.

Although our analysis predicts more pupae than observed adults, it is probably premature to say larval host is not limiting given several assumptions made that could be inflating our predicted values. Larvae were fed in small cups under lab, not field conditions. Thus our leaf consumption estimates may be lower than for free ranging larvae under fluctuating, and colder, field temperatures. In addition, using the native host could increase consumption estimates if larvae need to consume more non-native host to overcome different levels of chemical defense or extract sufficient nutrients. However, using the non-native host could decrease estimates if the native host is more defended or of lesser nutritive value. The fact that larvae of *Speyeria* species often feed on multiple hosts [8,38–41] and can be reared on non-native hosts including commercial pansies [23,38,41] makes it difficult to know how problematic this is and it should certainly be investigated further.

Additional assumptions regarding larval movement emphasize how the difference between amount of host *present* and amount *available* impacts estimates of population size. Our simulation analysis assumed that larvae find and consume every plant. This is not likely given the poor host finding ability documented in other *Speyeria* [30]. Furthermore, finding every plant assumes unlimited larval movement. In effect, host finding limitations decrease the amount of host *available* to larvae, making it much less likely that larvae find small, distant patches of host. For example, assuming the average transect density of 0.037 *Viola* per m^2 and uniform density, together with average values for number of leaves per plant (=10.3) and average leaf area (=1.99 cm^2), there is 0.76 cm^2 food per m^2 and based on our consumption estimates an average male would need 260 m^2 of area to reach pupation, and a female 384 m^2. These large areas highlight how potentially unavailable the host present may actually be.

In addition to *Viola* abundance, biomass and density, there are other aspects of *Viola* ecology that may be involved in "bottom–up" interactions that help explain the discrepancy between predicted pupae and adult estimates here, and declines in *Speyeria* in general. Host distribution is a potentially very important variable that can affect larval mortality. For example, the simulation study of Bierzychudek and Warner [31] showed that in addition to density, spatial distribution influenced pupation probability, and host finding success improved when *Viola* were arranged as randomly spaced clumps. In our study, *Viola purpurea quercetorum* on Chews ridge are not in dense carpets like some other *Viola* (e.g., *V. pedunculata*), but are heterogeneously distributed, with the densest patches widely separated (Figure 2). If we account for host distribution in our estimation of total number of pupae by estimating the number of transect segments 30 m in length that have enough plants to support a pupa (10 or more based on our estimated consumption rates if we assume average leaf area), there are 34 such transect segments (22,260 m^2 sampled). Since the area sampled represents 9.2% of the total study area, then a naïve extrapolation would yield 371 supported pupae. This value is within the 95% CI of estimated number of adults in 2013.

It may also be important to account for competition that results from the clumped distribution. If females preferentially lay eggs near appropriately sized clumps of host, rather than seeking isolated

plants, it follows that many larvae are present in a clump. Although we did not observe larval feeding behavior in the field, it seems reasonable that *S. adiaste* are similar to other *Speyeria* and stay near a plant they are consuming [31], and are unable to locate hosts from more than a few centimeters away [30]. Assuming these aspects of *S. adiaste* larval ecology are true, the implication is that larvae may need to leave a clump as a result of scramble competition. This exposes larvae to starvation as they seek additional hosts and reduces the number of eclosing adults compared to amount of host present.

Finally, induced defenses in *Viola* hosts could make the host biomass *present* unequal to the host biomass *available* or useable by larvae. Even if a host is easily found by a larva, some leaves or individuals may be poor quality or inedible because of previous herbivory [42]. A study with spider mites and pansies (*Viola* × *wittrockiana* Gams) showed that inducing plant defenses with methyl jasmonate caused the herbivores to disperse more quickly from treated plants [43]. Several *Viola* species are documented to have cyclotides [44–49], proteins which have been shown to provide defense against lepidopterans [50–52] and to have antimicrobial activity [46]. If these defenses are generally applicable, *Viola* that have been eaten by *Speyeria* may have higher concentrations of herbivore defenses that make the individual leaf or plant less palatable or even deadly. Observations in the field seem consistent with this, in that during the surveys for this project we observed only part of a leaf or one leaf and not an entire plant eaten by *Speyeria adiaste* (RIH and CER personal observation). This could indicate larvae eat one leaf and move on, but field observations are needed to confirm this since the pattern may result from smaller larvae sheltering nearby and returning over and over to eat.

The differences we observed in male and female consumption have implications for female fecundity, sex ratio and how many adults can be sustained. Males consumed significantly less than females, and males were significantly smaller than females in both the lab and field samples. For males the small size and lower leaf consumption may relate to selection for protandry—males eclosing earlier have access to the first females eclosing. However, this may be counteracted by male-male competition where larger males or males with larger thoraxes win competitive bouts for hilltops. For females, the association of higher leaf consumption and larger size should relate strongly to fecundity, as fecundity is affected by food limitation in *Speyeria mormonia* [53], and is positively correlated with adult size among butterfly species [54]. However, no data on the relationship between female fecundity and body size or leaf consumption are available for *S. adiaste*, and few other *Speyeria* have been studied. *Speyeria idalia* females are smaller where host density is lower [8], and in *S. mormonia* food limitation affects number of eggs [53], but body size does not [53,55], although very large females lay larger eggs [55]. If food limited or smaller females do lay fewer eggs (or smaller eggs) then this represents a food limitation mechanism for population declines other than larval mortality. In addition, the number of males that can be sustained is larger than the number of females given that females consume more host leaf. Furthermore, if plant density or spatial arrangement are suboptimal, this may have a disproportionate effect on female mortality, reducing female numbers and contributing to male biased adult sex ratios and population declines.

Although food resources are obviously important for herbivores and have been shown to be important in this study for *S. adiaste*, top–down interactions from predators and parasites may also be important. Little is known about the parasites of eggs, larvae and pupae of *Speyeria*, but *Madremyia* tachinid flies are likely important larval parasitoids given their wide Lepidoptera host range, and documentation on multiple *Speyeria* [56,57]. Predators likely play a significant role in larval mortality in *S. adiaste* and *Speyeria* in general. For example, Bierzychudek and Warner [31] observed predation attempts by ants and folding-door spiders (*Antrodiaetus* sp.) on *S. zerene hippolyta* searching for host plants in the field. Bierzychudek and Warner [31] stated that predation risks increase with more time looking for host plants. This predation risk would be higher in lower densities or spatial arrangements requiring larvae to travel farther. Predation would also likely affect female larvae disproportionately and in turn the adult sex ratio, given that females require more host plant and presumably move more to find it. Learning more about parasitoids and predators of *S. adiaste* would be worthwhile and given

the difficulty finding larvae in the field [23] methods similar to Bierzychudek and Warner [31] using lab reared larvae could provide useful data on predation and possibly parasitism rates.

Our approach provided a detailed snap shot of a single *Viola purpurea quercetorum* population at a local scale, but like most methods it has benefits and limitations. Limiting assumptions have been discussed above, but a benefit of this local scale approach is that it provided more detailed data on host plant ecology and larval food limitation compared with correlations of host abundance among many populations. As such, the distributions and summary statistics of plant traits and larval consumption can be useful in calculating target population sizes of *Viola* or *Speyeria*. The approach taken here is also useful for depicting variation in the system by incorporating stochastic rather than deterministic values for different variables. For example, drawing from the distribution of leaf area consumed that included both males and females included a degree of realism given that leaf area consumed will depend on larvae finding the hosts and our data were for larvae fed *ad libitum*. With more data on leaf consumption it would be possible to simulate males and females separately, but they share the resource so assumptions about sex ratio would need to be made.

As mentioned in the above paragraphs, there are many opportunities for further research in this system. One important area is to obtain data on leaf consumption on native larval hosts to investigate variation on different native hosts, as well as to measure size of resulting adults and female fecundity. It would also be interesting to investigate larval behavior consuming plants that have or have not been partly eaten by larvae. The excess host observed in our study is a good thing because it indicates larval resources are present to support more butterflies in some years—but temporal variation needs to be investigated. Replicating this study at a smaller scale within the Chews Ridge study area across years would help clarify the food limitation hypothesis. For example, tracking abundance in the densest patches and using the distributions for other plant traits could demonstrate a correlation with adult counts, as well as any discrepancies between predicted number of pupae and adult counts. Studying *Viola* and *Speyeria* at additional sites could also provide valuable data on natural *Viola* spatial distribution and density to know whether a site could support more butterflies given the host present.

5. Conclusions

Our results strongly corroborate and complement research on host–butterfly interactions in other *Speyeria* species providing useful data for management and restoration. Monte Carlo simulations indicated that the adult population estimate fell in the first quartile of what can be expected based on host density and biomass *present* at the site. Although these results suggest at face value that the *S. adiaste* population was not food limited, they should be interpreted carefully because the analysis included several assumptions that would lower the actual number of butterflies supported. Overall it would be reasonable to expect a few hundred, but certainly not more than one thousand adult *S. adiaste* based on host resources *present* in the study site. Although more information is needed on larval behavior of *S. adiaste*, and other *Speyeria*, and on how different *Viola* are distributed, results of this study and other recent work provide useful guidelines and tools for management and restoration of *Viola* food plants. For example, the models here can be used to understand whether larvae in a population appear food limited by calculating the number of adults that could be theoretically sustained by the host density present. Conversely, they can also be used in calculating the density of host required for a target number of adults. Even with the assumptions of our study, the amount of "excess" host observed in our analysis indicates that to achieve a robust population for a target number of butterflies, the *Viola* density should be twice or more what is required based on mere biomass. In addition to ensuring density is more than adequate, randomly spaced clusters of plants will likely increase the probability of encountering another plant before larvae starve or are predated [31]. Management and restoration of *S. adiaste*, and other *Speyeria*, should focus on not only maintaining excess levels of density, but also on the spatial distribution of larval food plants.

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draft preparation, R.I.H.; writing—review and editing, R.I.H., C.E.R. and J.M.; supervision, R.I.H.; project administration, R.I.H.; funding acquisition, R.I.H. and C.E.R.

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Appendix A

Truncated normal distribution R code.

We called it dtnorm2 to differentiate between the packages dtnorm():

```
dtnorm2<- function(x, mean, sd, a, b) {
dnorm(x, mean, sd)/(pnorm(b, mean, sd)-pnorm(a, mean, sd))
}
ptnorm <- function(x, mean, sd, a, b) {
(pnorm(x,mean,sd) - pnorm(a,mean,sd)) /
(pnorm(b,mean,sd) - pnorm(a,mean,sd))
}
```

Appendix B

Results of model fitting for the number of leaves per plant, leaf area per leaf, and leaf area consumed datasets.

Table A1. Number of leaves per plant (*V. purpurea*).

Distribution	Likelihood	AIC
log-normal	−778.7	1561.5
gamma	−780.2	1564.4
weibull	−784.2	1572.3
trunc-normal	−793.5	1590.9
normal	−841.0	1686.0

Table A2. Leaf area per leaf (*V. purpurea*).

Distribution	Likelihood	AIC
gamma	−2107.8	4219.5
weibull	−2108.5	4221.0
trunc-normal	−2119.5	4242.9
log-normal	−2122.9	4249.7
normal	−2138.3	4280.6

Table A3. Leaf area *V. papilionacea* consumed.

Distribution	Likelihood	AIC
weibull	39.3	−74.6
normal	38.7	−73.4
trunc-normal	38.7	−73.4
gamma	38.3	−72.6
log-normal	38.0	−72.0

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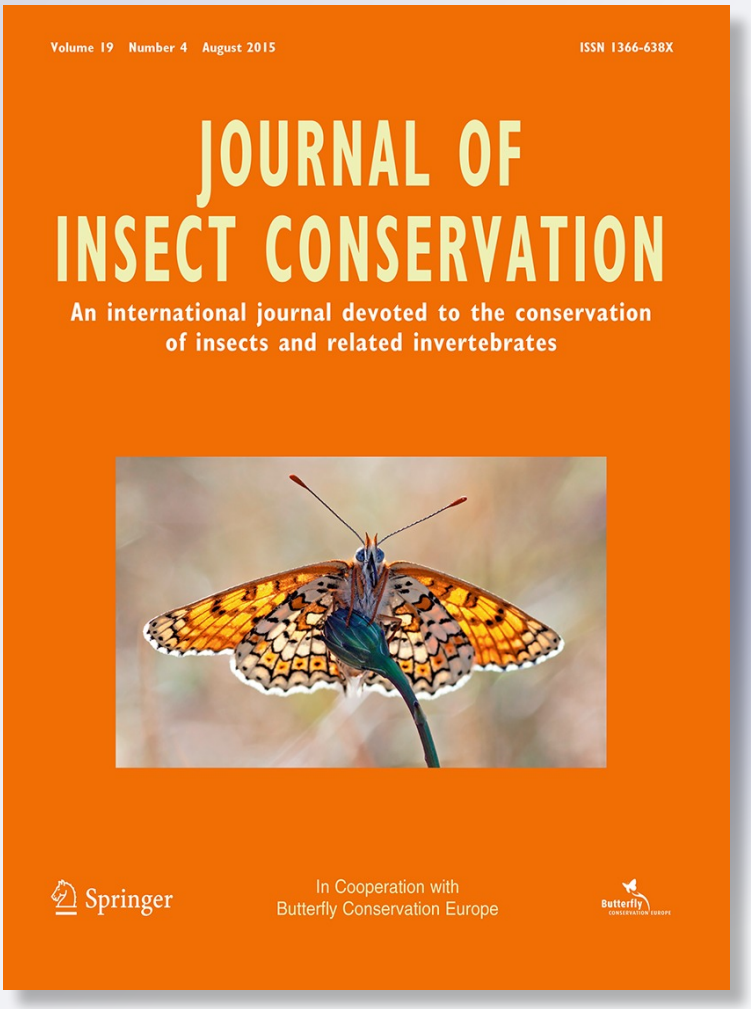

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ORIGINAL PAPER

Population ecology of a California endemic: *Speyeria adiaste clemencei*

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Abstract Effective management and recovery of threatened insect populations requires detailed ecological information. Here we combine count and mark recapture (MR) data to shed light on an understudied declining endemic butterfly species in California's Southern Coast Ranges, *Speyeria adiaste*. Little is known about the number, size, and dynamics of *S. adiaste* populations, leaving few data on which to base conservation decisions. Our goal in this study was therefore to provide increased understanding of the population ecology of this species by studying a long-standing *S. adiaste clemencei* population. Our 2-year MR estimates were highly correlated with Pollard walk counts, and we observed declining population sizes from 2011 to 2014. Adult movements were well-described by a negative exponential function, indicating low probability of dispersal >5 km (probability $< 1.8 \times 10^{-7}$ for both sexes). Males had shorter lifespans than females. Coupled with lack of diapause in this species, the short life span and limited dispersal observed here indicate that *S. adiaste clemencei* does not have a strong capacity for re-colonization. Population declines in *S. adiaste* may lead to local extinctions, and together with low dispersal, may diminish connectivity among the apparently small and isolated remaining populations. Further research into the role of adult and larval resources for determining adult abundance, coupled with continued long-term monitoring is necessary in order to understand population dynamics in this declining endemic species.

Keywords Demography · *Viola purpurea* · Nymphalidae · Mark-recapture

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Introduction

Effective management and recovery of endangered species requires detailed biological knowledge of individual species. For example, recovery plans need objective data such as population sizes, growth rates and number of subpopulations to use as criteria for change (Schultz and Hammond 2003). Gardmark et al. (2003) concluded that in the majority of cases they reviewed, recovery is determined by a single life history trait or interaction, but "ecologists need to be open-minded about what kind of life-history trait or interspecific interaction could be the most important in any particular case". Thus, a broad and thorough knowledge of a study species can provide the potential clues to effective management. Insects, however, are relatively understudied (New 1995; Schultz and Chang 1998), limiting the data on which to guide management decisions for species in this numerically dominant and ecologically important group. Furthermore, obtaining the detailed biological information required for management may be difficult or impossible once a species declines substantially.

In this paper we investigated the population ecology of the Unsilvered Fritillary butterfly, *Speyeria adiaste*, a species that has been denied listing under the endangered

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species act because of a lack of data on the size, distribution, and number of extant populations (USFWS 2011). Among butterflies in the genus *Speyeria*, *S. adiaste* is one of the most geographically restricted species (Fig. 1), and has been reportedly declining throughout its range (Scott 1986; Opler and Wright 1999; Glassberg 2001; Kaufman and Brock 2003; Shapiro and Manolis 2007). In fact, the southern-most subspecies *S. adiaste atossa* is extinct, making this the only *Speyeria* species to have a described subspecies go extinct (but see *S. zerene* in Shapiro and Manolis 2007). The remaining two subspecies, *S. a. adiaste* and *S. a. clemencei*, are ranked as critically imperiled to imperiled, but are not currently listed under the State or Federal Endangered Species Acts (USFWS 2014).

Motivated by the relative paucity of information about this species, we undertook a study of *S. a. clemencei* in Monterey County, California. *S. adiaste* is restricted to the Southern Coast Ranges of California in mixed oak-pine woodlands (Zaman et al. 2014, and references within). Like all *Speyeria*, *S. adiaste* is a univoltine species in which the first instar overwinters. The larvae commence eating their *Viola purpurea* hostplant (Scott 1986; Zaman et al. 2014) in the spring and adults are found on the wing in early to mid-summer. Zaman et al. (2014) found pronounced differences in male and female behavior, with males spending more time in flight-related activities compared to females. Unlike females of some *Speyeria* that exhibit reproductive diapause, in which the females mate but do not begin laying eggs for several weeks (Sims 1984; Shapiro and Manolis 2007), *S. adiaste clemencei* were observed ovipositing relatively soon after eclosing.

The behavioral and life history traits of *S. adiaste* have implications for its dispersal propensity and population structure. Between sexes of *S. adiaste clemencei*, the increased flight activity for males predicts increased mortality risk and shorter life spans, and increased dispersal compared to females. Compared to *Speyeria* species that do have reproductive diapause (Sims 1984), the lack of reproductive diapause in female *S. adiaste clemencei* predicts relatively short life spans and lower dispersal propensities. Since dispersal is a critical aspect of metapopulation dynamics that can influence persistence of patchily distributed subpopulations (Hanski 1985; Gustafson and Gardner 1996; Cronin 2003), increased understanding of these aspects of *S. adiaste* population ecology can shed light on colonization dynamics and inform conservation decisions.

Speyeria adiaste appears to exist in relatively widely spaced colonies, implicating dispersal as a key for re-colonization to maintain gene flow and allow re-colonization of isolated habitat patches. If *S. adiaste* is a widely dispersing species that has high rates of colonization then it

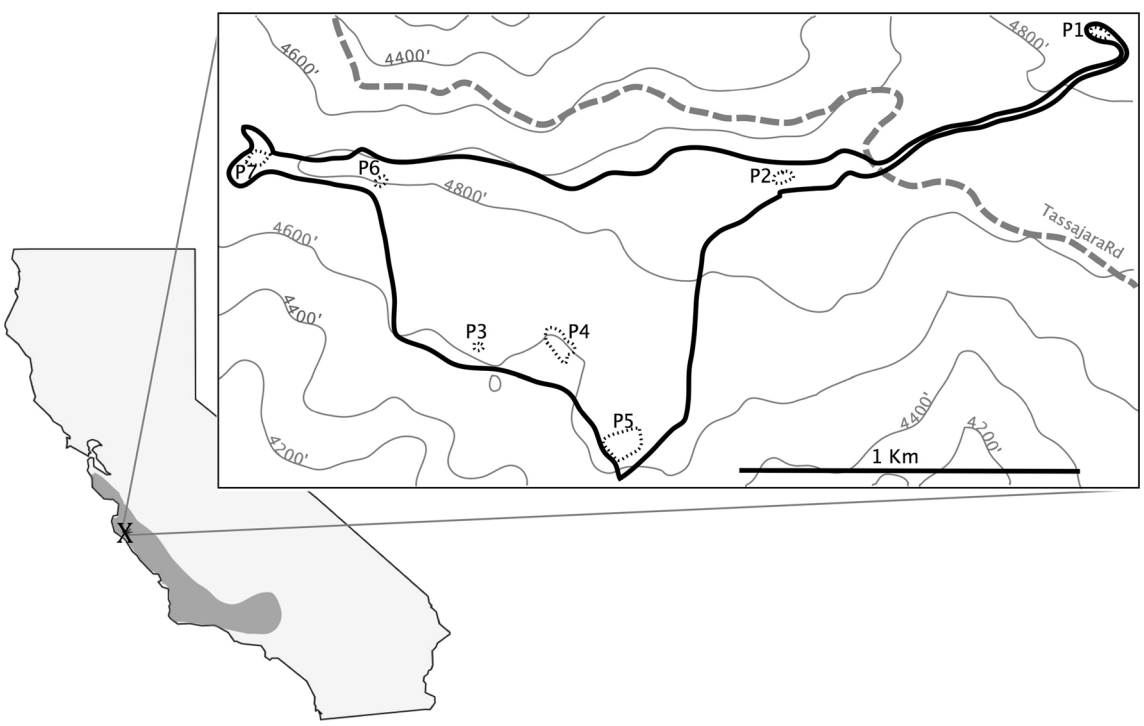

Fig. 1 Range of *S. adiaste* and map of the Chews Ridge study site (*bold line*). Chews Ridge is marked on state map with an X. Study site elevation: 1447–1541 m. Total study site area was 243,207 m^2. *Plot 1 area* 1103 m^2, *Plot 2 area* 192 m^2, *Plot 3 area* 391 m^2, *Plot 4 area* 2293 m^2, *Plot 5 area* 1599 m^2, *Plot 6 area* 384 m^2, *Plot 7 area* 690 m^2

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may be able to persist in widely spaced colonies in a fragmented landscape. However, if it is highly philopatric with low dispersal, in combination with the observed lack of reproductive diapause, this could lead to increased risk of local extinction with little chance of re-colonization. In this latter case, management to increase population size in key populations, coupled with reintroductions to invigorate isolated colonies, may be necessary to conserve the species. Accordingly, in this study we aim to provide a better understanding of *S. adiaste* and its population ecology by combining count-based methods and mark-recapture methods to estimate population size, survival, dispersal and fluctuations in abundance.

Materials and methods

Study site and sampling design

This study focused on the *S. adiaste clemencei* population at Chews Ridge, Monterey County, CA (N36.31336°; W121.57323°, 1500 m elevation). *S. a. clemencei* is found in the Santa Lucia mountains in Monterey and San Luis Obispo counties and is not known to be found below 1067 m (3500 ft) elevation (Howe 1975). The total study area at Chews Ridge, incorporating all plots and areas where butterflies were captured or counted was 243,206.5 m^2 (Fig. 1).

Observations were made from 2011 to 2014. Pollard walk counts were conducted weekly from May to August 2011–2014. To standardize the counts, the same route was always used, counts were made by a single observer (C. Tenney) walking at a steady pace, and only butterflies in front of the observer were counted. During counting any butterfly that could be positively identified was included. Counted individuals were not sexed owing to difficulty in positively determining sex on the wing. To associate counts with mark-recapture estimates, the counts were done on the first day of the mark-recapture study each week.

Mark-recapture data were collected two consecutive days per week in 2012 and 2013. Mark-recapture sampling began the week following the first appearance of butterflies during the Pollard walk count. In 2012 sampling was conducted across 10 weeks starting June 13th and ending August 23rd with one week skipped because of logistical issues. In 2013, sampling was conducted for eight consecutive weeks from June 5th to July 25th. Each day, sampling started at 10 am and ended approximately 3–4 pm in the afternoon when butterflies were no longer seen flying.

For the mark-recapture study seven plots within the study area were established to standardize sampling effort across the site (see Fig. 1 legend for specific plot sizes). The particular sites selected were chosen to maximize the amount of butterfly sightings, and represent 2.7 % of the total study area. The GPS location of each plot was taken approximately in the center, which then became the default GPS location data for all butterflies captured within each plot. The order in which plots were sampled was chosen haphazardly each day. Two collectors spent 30 min marking butterflies within each plot on each sampling day. After each 30 min session, the collectors walked between plots and during this time any butterflies seen were also captured and GPS data were recorded.

Observations in 2011 indicated that ridge sites were used by males as hill-topping sites, whereas meadow sites were used as oviposition sites by females. Thus four of the plots were relatively higher elevation ridge sites ranging from 1447 to 1541 m (plots 1, 2, 6, 7). The remaining three plots were lower elevation meadow sites ranging from 1401 to 1408 m (plots 3, 4, 5). Chi square tests using three ridge sites and three meadow sites for each sex were used to test the null hypothesis that individuals were distributed equally among ridge and meadow sites. Site 1 was left out in this test because it was far removed from the other sites and had no paired meadow below it where females could easily move up and down slope.

During mark-recapture sampling butterflies were captured and released in a way to reduce the effects of handling. Butterflies were caught with aerial nets and during plot sampling were held in mesh cages in the shade until sampling ended. Butterflies caught outside of plots were released immediately by placing them gently on a leaf or branch near the point of capture. Each individual captured was marked on the underside of both hind-wings with an individual number using an ultrafine point Sharpie pen. We did not observe adverse effects of marking and handling on the butterflies, as the butterflies resumed their behavior prior to being captured after being released. The time of capture, date, wing wear, behavior, and sex of the butterfly was recorded.

Demographic and population size analyses

We used two modules within program MARK (White and Burnham 1999) which are suitable for open populations with births, deaths, immigration, and emigration. To estimate overall population size the Jolly–Seber POPAN (JSP) parameterization of the Jolly–Seber method was used. The Robust Design module in program MARK (RDM) was also applied to obtain week to week population size estimates and estimates of demographic parameters. We obtained an estimate of residence time using the equation—$\ln(\Phi)^{-1}$ (Watt et al. 1977) where S from the RDM model was substituted for Φ in the Watt et al. (1977) equation. For a description of model parameters and comparison of parameter estimates between the JSP and RDM models, see the supplementary material.

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For both JSP and RDM, model fitting began with the simplest models possible, using parameters constant in time and no group (i.e. sex) differences. More complexity was added and each model's fit was determined based on decreasing values of Akaike's Information Criterion adjusted for small samples sizes (AICc) (Burnham and Anderson 2002). A difference in AICc (ΔAICc) of greater than two between models was used as evidence for significantly improved model fit (Burnham and Anderson 2002). The best models based on AICc values were then checked for logical parameter values, and models with confounded or inestimable parameters were not considered. Thus the model with the lowest AICc that gave realistic estimates, with non-confounded parameters was chosen.

Correlation of MR estimates with Pollard walk counts

To assess the correlation of counts and actual abundance we compared the weekly Pollard walk count data and the weekly estimates of RDM population size (N_i) (see supplementary material for correlation with estimates of JSP $\hat{N}_i$). In order to make a better comparison of the results of the Pollard walk counts and the mark recapture (MR) estimates, capture histories were analyzed with group (sex) removed to obtain overall population estimates week to week. This was done because during the Pollard walks, sexes were not distinguished. This MR model is referred to as RDM-N, with "-N" in the model name indicating models without sex. We used R (R Development Core Team 2014) to calculate the Pearson product moment correlation and its significance (cor.test), and tested for a difference in correlation coefficients between models with Fisher's z-transformation (Fisher 1915), and for a difference in fitted slopes using Student's T (Student 1908).

Dispersal analysis

A summary of lifetime movements for each individual and estimates of dispersal were obtained by summing distances between recaptures for individuals that were recaptured at least once. Probability of dispersal was estimated using the inverse power function (IPF) and the negative exponential function (NEF) (Hill et al. 1996; Konvička et al. 2005). The IPF function (Eq. 1) and NEF function (Eq. 2) express the probability density I of movements to distances D in kilometers.

$$I_{IPF} = C \cdot D^{-n} \quad (1)$$

$$I_{NEF} = a \cdot e^{-kD} \quad (2)$$

To estimate the values of the parameters (a, k, C, and n), each function was fitted by regressing the natural logarithm of the cumulative fractions of individuals moving to distances D or greater (ln I) against linearized expressions of the distances, i.e. $\ln C - n \cdot (\ln D)$ for IPF and $\ln a - kD$ for NEF. Predicted values of I for each model were compared with the observed cumulative proportion of the movements using least squares. Model selection was again based on AICc values, with a ΔAICc value greater than two indicating a better fit. Analyses were done in Microsoft Excel and Graphpad Prism 6. Model fitting and selection was carried out for both sexes separately.

To investigate the association of local host abundance with habitat use we quantified *Viola* hostplant abundance on the study plots. The abundance of *V. purpurea* was estimated at each of the study plots using transects spaced every 4 m. Transects were split into 30 m segments and all *V. purpurea* individuals within 1 m of each side of the transect line were counted. *V. purpurea* cover area was obtained by multiplying the longest ground projected diameter of a *Viola purpurea* individual, or group of individuals, by the distance perpendicular to that longest diameter. The Pearson product moment correlation (cor.test) between butterfly abundance and host abundance was calculated in R (R Development Core Team 2014). A one-tailed P value was used given the a priori expectation of increased butterfly abundance with increase host.

As part of an investigation into mitochondrial DNA variation in *Speyeria* butterflies in the Bay Area, the sequence has been generated for *Cytochrome oxidase subunit I* (~884 bp. *CoI*) for 33 *S. adiaste* individuals (see Table S4). A piece of hindwing tissue from each wing was removed and stored in 95 % ethanol for DNA extraction using a Qiagen DNeasy kit. Primers (Lep3.1F/R) and PCR conditions follow Dasmahapatra et al. (2010).

Results

Mark recapture

Over the course of the 2-year MR study, a total of 392 individuals were marked with 186 (47.4 %) individuals recaptured (Table 1). In 2012, 325 individuals were marked, 252 males and 73 females. Of the 325 individuals, 131 males and 30 females were recaptured, for a combined 161 recaptured individuals (49.5 %). Males had a higher recapture rate (52 %) compared to females (41.1 %) in 2012. In 2013, there was a large reduction in the total number of marked individuals ($N = 67$), compared to 2012. Only 51 males and 16 females were marked in 2013.

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Table 1 MR summary data from 2012 to 2013

	Individuals marked	Individuals recaptured	% Individuals recaptured	Total capture events	Total recapture events
2012					
Males	252	131	52.0	480	228
Females	73	30	41.1	112	39
Total	325	161	49.5	592	267
2013					
Males	51	23	45.1	82	31
Females	16	2	12.5	18	2
Total	67	25	37.3	100	33
2012–2013					
Males	303	154	50.8	562	259
Females	89	32	36.0	130	41
Total	392	186	47.4	692	300

Note that total capture events columns include handling individuals multiple times

Males were recaptured more often (45.1 %) than females (12.5 %) in 2013.

Population size and fluctuations

The overall population size for each sex was obtained from the JSP model estimates (see Table S1 for the specific model). In 2012, the estimated population size was 531 individuals (♂ 362, 95 % CI 324–420; ♀ 169, 95 % CI 128–240, see Table S3). The total population size declined in 2013, with an estimated 151 total individuals (♂ 115, 95 % CI 80–190; ♀ 36, 95 % CI 24–66).

Pollard walk counts from 2011 to 2014 revealed that the flight season started one week earlier in 2012 and two weeks earlier in 2013 and 2014 when compared to 2011 (Figure S3). The timing of the peak count was also observed to be two weeks earlier from 2011 to 2012, and from 2012 to 2013, with 2014 being one week earlier than 2012. The 2011 flight season lasted 12 weeks, the 2012 season lasted nine weeks and the 2013 and 2014 flight seasons only lasted about eight weeks. The peak count in each year, as well as the sum of all counts, decreased each subsequent year after 2011 (Figure S3). RDM-N weekly population estimates (Table S3; Figure S4) were significantly correlated with the weekly Pollard counts (Fig. 2; $r = 0.73$, $t = 4.3$, $P = 5.4 \times 10^{-4}$) indicating that actual butterfly abundance is 3.9 times the number of counted individuals (see supplementary materials for discussion of JSP-N results).

Demography

Results of the best fitting JSP and RDM models were very comparable and we only discuss the RDM results below because the week-to-week population estimates appear more realistic and RDM provides additional information. For discussion of JSP parameters and differences between the models see the supplementary materials.

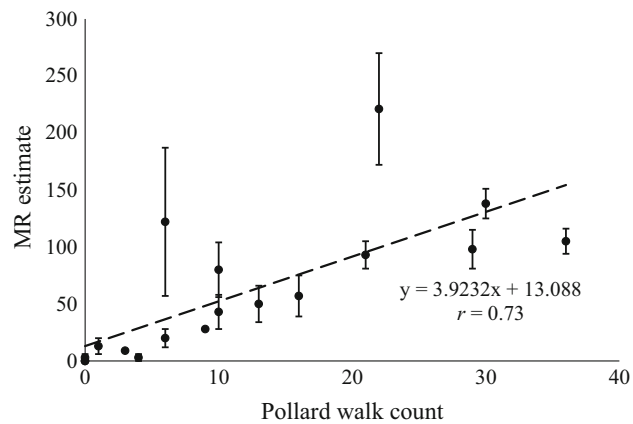

Fig. 2 Correlation of weekly Pollard walk counts with MR estimates. The RDM model showed a significant correlation with count data from 2012 to 2013

The best fitting RDM model for 2012 (See Table S1 for the specific model) revealed that weekly survivorship S was constant and higher in females ($S = 0.61$, 95 % CI 0.47–0.74) than in males ($S = 0.49$, 95 % CI 0.44–0.55). Calculated average residence time (Watt et al. 1977) was 10 days for males (95 % CI 9–12 days) and 14 days for females (95 % CI 9–23 days). For both males and females, emigration (γ'') and immigration (γ') rates were equal but differed between sexes (♂ 0.28, 95 % CI 0.16–0.44; ♀ 0.64, 95 % CI 0.45–0.80). Males and females had an equal and constant capture probability ($P = 0.54$, 95 % CI 0.45–0.62), and an equal and constant recapture probability ($c = 0.23$, 95 % CI 0.18–0.28).

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In 2013 when the population size was much smaller, model averaging was used for the top two RDM models to obtain parameter estimates because of their very similar AICc scores (see Table S1) (Burnham and Anderson 2002). Weekly survivorship was constant and equal for both sexes ($S = 0.54$, 95 % CI 0.31–0.75). Calculated average residence time was 11 days for both sexes (95 % CI 6–25 days). The emigration probability γ'' was constant and equal for both sexes ($\gamma'' = 0.81$, 95 % CI 0.60–0.93). Immigration probability γ' was also constant and did not differ between sexes ($\gamma' = 0.65$, 95 % CI 0.16–0.95). Capture probabilities were constant and did not differ between sexes ($P = 0.62$, 95 % CI 0.46–0.75), recapture probabilities were also constant and did not differ between sexes ($c = 0.19$, 95 % CI 0.10–0.32).

The curves of sex-specific weekly population sizes from the RDM model revealed strong protandry (Fig. 3). In 2012, males peaked in week 2 (Fig. 3a). In 2012 females peaked in week 5, and became more abundant than males from week 6 onward. In 2013, males and females peaked in week 5 (Fig. 3b), protandry was less pronounced, and male numbers remained above females throughout the entire flight season.

Genetic diversity

Four *CoI* haplotypes were found among the 33 sequenced individuals (Supplementary Figure S7 and Table S4). *S. adiaste clemencei* samples were represented by three haplotypes. The Chews Ridge population ($N = 26$) had all three haplotypes present and was comprised almost entirely of two haplotypes (12 and 13 individuals respectively), with the third haplotype being represented by a single individual. Sampling of other Monterey Co. populations was limited but did not add any additional haplotypes. Four individuals from the West Ridge site, near Chews Ridge, were all identical. The two *S. adiaste adiaste* individuals were identical, and differed by four base pairs from the Monterey Co. samples.

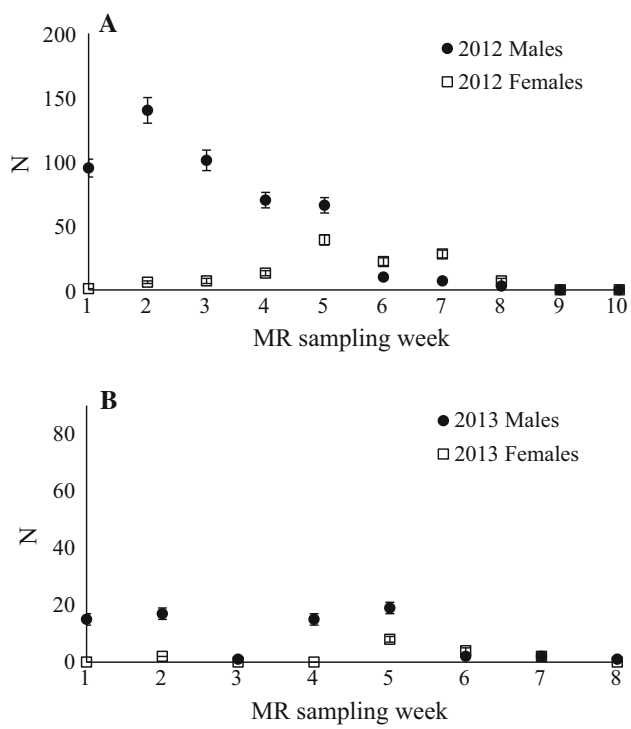

Fig. 3 Weekly RDM model population size estimates. **a** 2012 RDM model estimates. **b** 2013 RDM model estimates

Habitat use and dispersal

Males were found in equal numbers on ridge and meadow plots ($X^2 = 2.2$, $P = 0.13$), whereas females were found more often on meadows than ridges ($X^2 = 33.9$, $P = 5.8 \times 10^{-9}$). The number of female captures on each plot was significantly positively correlated with estimated *Viola* cover on each plot ($r = 0.74$; $P = 0.028$). However, total butterfly abundance ($r = 0.33$; $P = 0.23$), and male abundance ($r = 0.26$; $P = 0.28$) were not correlated with *Viola* cover (Figure S6).

Dispersal differences were evident between the sexes. Males and females did not differ in mean total observed distance moved (Mann–Whitney, $U = 2114$, $Z = 0.42$, $P = 0.67$) (Table 2). However, for both sexes, the NEF model was a better fit than the IPF model (Table 3) and the slope of the linearized NEF function differed between the sexes (F test, $F = 48.5$, $Dfn = 2$, $Dfd = 28$, $P < 0.0001$) indicating higher probability of male dispersal, especially at larger distances.

Discussion

Population size and declines

Our analyses clearly indicate that populations of *S. adiaste clemencei* are not very large. The best fitting population model for the MR data (see Table S1) estimated overall population size at Chews Ridge to be 531 in 2012 and 151 in 2013. Total cumulative counts in any season never reached 300 individuals, and the average yearly total during the 4-year period was 136 counted individuals, with yearly counts ranging from 46 to 282 individuals. *S. adiaste* was less abundant than sympatric populations of *Speyeria callippe* and *S. coronis* at Chews Ridge during the study period (C. Tenney and R.I. Hill pers. obs.), and the Chews Ridge colony was the largest found during surveys conducted across Monterey Co. (see supplementary materials).

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Table 2 Summary of *S. a. clemencei* movement data 2012–2013

	N	Mean single distance	Maximum single distance (m)	Mean total distance	Maximum total distance (m)
Male	154	145 m (SD 222 m)	1210	254 m (SD 305 m)	1570
Female	33	620 m (SD 157 m)	620	222 m (SD 173 m)	620

Table 3 NEF and IPF models for dispersal

Model	Equation	ΔAICc	250 m	500 m	1 km	5 km	10 km
NEF ♂	$\boldsymbol{P = \ln(0.95, \pm 0.054) – 3.1(\pm 0.074)D}$	**−57**	**0.44**	**0.20**	**0.043**	$\mathbf{1.8 \times 10^{-7}}$	$\mathbf{3.3 \times 10^{-14}}$
IPF ♂	$P = \ln(0.05, \pm 0.00077) – 1.4(\pm 0.14)\ln D$	–	0.32	0.12	0.048	0.005	0.002
NEF ♀	$\boldsymbol{P = \ln(1.97, \pm 0.31) – 5.9(\pm 0.41)D}$	**−21**	**0.45**	**0.10**	**0.005**	$\mathbf{3.0 \times 10^{-13}}$	$\mathbf{4.7 \times 10^{-26}}$
IPF ♀	$P = \ln(0.053, \pm 0.02) – 1.3(\pm 0.26)\ln D$	–	0.31	0.13	0.053	0.007	0.003

Bold indicates NEF had ΔAICc >2 and was therefore a better fit for both sexes. Probabilities of moving to certain distances are given for each model

This study also clearly detected declining trends in population size and fluctuations in timing of *S. a. clemencei*. Both MR estimates and count data showed more than a threefold decrease in population size from 2012 to 2013, with a nearly fivefold decrease in the number of estimated females (see Table S3 last column), and total Pollard walk counts had a sixfold decrease from 2011 to 2014 (Figure S3). In addition to the trends in decreasing size, there were also trends showing earlier start time, shorter length, and earlier time of peak counts for each flight season from 2011 to 2013, with 2013 and 2014 being very similar overall (Figure S3).

Together, the small population size and fluctuations observed here over this relatively short time span highlight the imperiled nature of this particular study population and species. Estimates of relatively small population size and the declining trend in *S. adiaste clemencei* represent a substantial risk of extinction from demographic or environmental stochasticity during low population phases. If the fluctuations at Chews Ridge represent the natural year-to-year fluctuations across populations in this species, as they appear to be in some other *Speyeria* species (Bouseman and Sternburg 2001; Vaughan and Shepherd 2005, for Idalia and Diana fritillaries respectively, but see Boggs 1987 for Mormon fritillary), then extinction of even large, long-standing populations such as was studied here may be expected (Hanski 1997). In this case, *S. adiaste* could be considered a metapopulation, although this could also be one large patchily distributed population (Hanski and Simberloff 1997; Harrison and Taylor 1997). Continued monitoring of this and other populations can help characterize dynamics among populations, such as relative size and whether they are in synchrony (Hanski 1997). In either case, dispersal is clearly a key for population and species persistence (see below) given the large distances between subpopulations (see supplementary materials).

Pollard walks and correlation with MR data

The strong correlation between MR estimates and count data give a rough calibration of actual abundance per count, providing a useful tool for monitoring *S. adiaste* populations. The data indicate one should not expect dozens of butterflies present per individual detected during a count, but rather four to six individuals per counted butterfly for the RDM-N and JSP-N models respectively based on the slopes (Fig. 2, Fig. S5). A calibration based on a single model is not supported because neither the correlation coefficients ($z = 1.6$ and $P = 0.10$), nor slopes ($t = 1.8$ and $P = 0.08$) differed between models (see supplementary materials). Furthermore, the RDM-N model slightly overestimates actual abundance relative to the JSP-N model at counts <6, and underestimates actual abundance at counts >6 (Figure S5). Thus, the correlations provide a rough guide linking counts to actual abundance.

The fact that the best-fitting RDM model in each year indicated constant survivorship and constant capture probabilities (see Table S1), gives us some confidence that counts from continued monitoring can be appropriately used for understanding actual population size fluctuations at Chews Ridge. The observed constant survivorship and constant capture probabilities remove potential bias in the association of abundance and counts (Haddad et al. 2008; Pellet et al. 2012). Caution should however be used in trying to extrapolate our results to other *S. adiaste* populations due to potential differences in habitat, population ecology, and observer identity, which can lead to biased estimates of abundance (Haddad et al. 2008; Pellet et al. 2012).

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Demography

Speyeria adiaste clemencei at Chews Ridge exhibit clear demographic differences between males and females. Strong protandry is evident in this species with males on the wing 2–3 weeks before females (Fig. 3), which is common in *Speyeria* (Boggs 1987; Kopper et al. 2001; James 2008) and other Lepidoptera (Neve and Singer 2008; Zwaan and Zijlstra 2008; Weyer and Schmitt 2013). The degree of protandry and peak of male and female abundance differed between years (Fig. 3) and could be explained by increased mortality of larvae that broke diapause early (i.e. males) and encountered dry winter conditions in 2013.

The male biased adult sex ratio and higher male recapture rates during the MR study (Table 1) may be attributed to behavioral differences combined with protandry. The increased flight behavior observed in males (Zaman et al. 2014) made them more conspicuous, probably increasing their capture/recapture rates. In contrast, female *S. adiaste* typically flew low to the ground, constantly landing and searching, with perching and searching for hostplants making up over 80 % of our observations during the course of the MR study (Zaman et al. 2014). However, in 2012, the year with adequate sample size, the RDM capture/recapture probabilities were equal for the sexes. This suggests that something other than differences in flight activity is needed to explain the observed sex ratio. Relatively few adult females could result despite an even sex ratio in the immature stages as a result of protandry (Scott and Mattoon 1981(82); Boggs 1987). With marked protandry as observed here, females must be spending more time in the immature stages, which could result in increased mortality of the larvae or pupae, leaving fewer females to eclose as adults. This potential increased mortality of immature females may be an important demographic variable limiting local population growth and is worth further investigation.

Results of this study indicate that *S. adiaste* adults are short lived and that as predicted, residence time and survivorship differ by sex. Our analyses indicate males have lower adult survivorship and residence times than females. The relatively longer female life spans can probably be attributed to both behavioral and life history differences between males and females. Longer apparent lifespans for females is common among Lepidoptera (Fric and Konvička 2000; Petit et al. 2001; Schtickzelle et al. 2002; Konvička et al. 2005). *S. a. clemencei* males engage in more conspicuous and potentially risky flight-related activities such as competing with other males for the best hill-topping/perching sites during mate location (Zaman et al. 2014). Differences in life history strategy between male and female *Speyeria* may also play a role. Boggs (1997) found that *S. mormonia* females needed extra time after eclosion to allow their ovaries and eggs to fully develop, which was facilitated by adult nectar intake. The potential additional developmental time combined with the life history strategy of *S. adiaste* females of laying eggs singly (Zaman et al. 2014) could select for a relatively longer lifespan than males (Boggs 1988), albeit not as long as in species that exhibit reproductive diapause (Sims 1984). In addition, female *S. adiaste* probably only mate once like *Speyeria mormonia* (Boggs 1986). This, coupled with the pronounced protandry (Fig. 3), indicates males should mate as soon as females are available, and that longer male residence late into the flight season may not provide additional male fitness benefits (Zimmermann et al. 2009). Although females have a longer lifespan than males, this can probably only add to local population growth due to lack of reproductive diapause (Zaman et al. 2014) coupled with the low probability of dispersal found in this study (see below).

Dispersal and habitat use

Our study indicated *S. adiaste* does not disperse long distances and that females have lower long distance dispersal potential compared with males (Fig. 4; Table 3). Both sexes have essentially equal probabilities of moving up to 250 m, well within the study area, but probabilities for females quickly decline afterwards compared to males (Fig. 4; Table 3). For example, the probability for males to disperse 5 km is 1.8×10^{-7} whereas for females it is 3.0×10^{-13} (Table 3).

The dispersal results are interesting given that females typically had a longer lifespan than males. Longer female lifespan could lead to increased time to move around and thereby increase their cumulative lifetime distance moved. Although the slopes of the NEF differed between sexes, the mean cumulative distance moved did not differ between sexes (Mann–Whitney, $U = 2114$, $Z = 0.42$, $P = 0.67$). The lower female long distance dispersal probability could have been due to the difference in sample size between the sexes ($N = 154$ males, $N = 32$ females). However, other differences correlated with movement were observed. The number of captures on ridge versus meadow sites indicates that females spend more time in and around meadows compared to males, who were more evenly distributed. Furthermore, the number of captures on each plot was correlated with the estimated *Viola* area on each plot for females, but not for males or when sexes were combined (Figure S6).

Together, our results indicate that female *S. adiaste* are philopatric and may be limiting their movements to stay near hostplants. The relatively low dispersal and female preference for nearby meadows rich in *Viola* hostplants may be driven by their life history strategy of laying eggs

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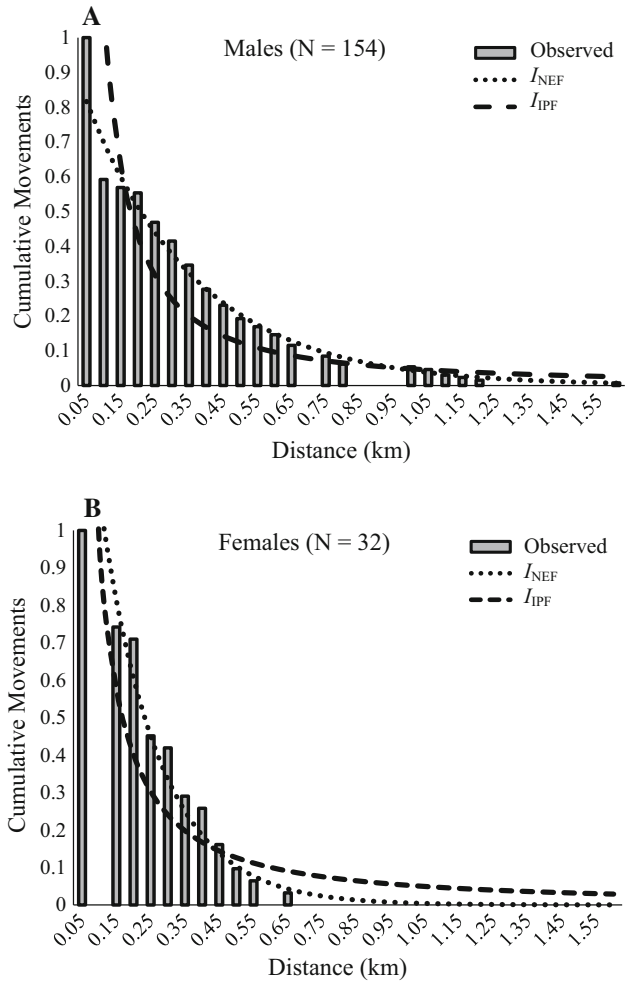

Fig. 4 Cumulative proportion of observed adult *S. a. clemencei* movements for males (**a**) and females (**b**) with fitted dispersal models. The negative exponential function model (NEF) was a better fit for both sexes than the inverse power function (IPF) model

relatively soon after mating (i.e. no reproductive diapause). *S. adiaste* females do not have reproductive diapause (Zaman et al. 2014) in contrast with the sympatric *S. coronis coronis* (R. Hill, pers. obs.). Populations of *S. coronis* that display reproductive diapause in California are known to have highly mobile and long-lived females as a result of this life history trait (Sims 1984; Shapiro and Manolis 2007).

The NEF function indicates that *S. adiaste* is a weak long distance disperser. However, Baguette and Schtickzelle (2003) in a study of long distance dispersal of *Boloria aquilonaris*, found that rare long distance movements could easily switch the relative fits of the NEF and IPF models. Other studies have shown that most MR studies are biased against long distance dispersal due to confining observations to just those within the study site (Baguette and Schtickzelle 2003; Franzen and Nilsson 2007; Zimmermann et al. 2011). Although this is not completely ruled out here, our results indicate that *S. adiaste* is prone to staying near the areas where they eclose. Only 7 of 186 recaptured individuals over the 2 year study moved a cumulative lifetime distance of at least 1 km and 94 of 186 moved a cumulative lifetime distance of 250 m or less. The study area was large enough to have observed movement distances greater than those we actually observed, giving us some confidence that we did not grossly underestimate movement distances.

Implications for population structure, re-colonization dynamics and persistence

The small observed population sizes and declines at Chews Ridge, coupled with large distances between populations and our estimates of short longevity and low probability of long distance dispersal, have important implications for the natural population structure and re-colonization dynamics for this species. Altogether, our data indicate that *S. adiaste* populations are groups of relatively isolated, closely related, philopatric individuals, and that dispersal is of critical importance for re-colonization and population persistence. It seems likely that subpopulations are prone to extinction and that re-colonization of vacant habitat patches occurs during phases of large population size when abundance can increase the probability of long distance dispersal.

If the decline to small population size (151 individuals, 36 females, in 2013) on Chews Ridge is representative of the dynamics across the *S. adiaste* range (Scott 1986; Opler and Wright 1999; Glassberg 2001; Kaufman and Brock 2003; Shapiro and Manolis 2007), there is probably prevalent genetic bottlenecking among populations (Saccheri et al. 1998; Nieminen et al. 2001; Harper et al. 2003; Willi et al. 2006). This would lead to naturally high levels of genetic differentiation among populations, and reduced genetic diversity within populations. The mtDNA data presented here are consistent with *S. adiaste* populations being isolated with low genetic diversity in having just two main haplotypes at Chews Ridge with one rare haplotype. In contrast, *S. callippe comstocki* and *S. coronis coronis* have seven and 11 *CoI* haplotypes at Chews Ridge respectively with similar sample sizes (R. I. Hill unpublished data).

The potential for reduced genetic diversity in widely separated *S. adiaste* populations could lead to reduced vitality associated with inbreeding (Willi et al. 2006; Saccheri et al. 1998; Nieminen et al. 2001). Low within-population genetic diversity could be counteracted through dispersal and gene flow. However our analyses indicate that this is not likely. The distances between current populations seem large enough (C. Tenney, K. Zaman and R. Hill pers. obs.) to make dispersal very rare, with a probability of 3.3×10^{-14} for a 10 km movement (Table 3). The low probability of long distance dispersal in *S. adiaste*

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clemencei, combined with life history attributes such as immediate oviposition (no female reproductive diapause), suggest that *S. adiaste* populations do not have a high natural propensity for re-colonizing vacant habitat patches. As a result of these aspects of the population ecology of *S. adiaste*, its long-term viability is certainly in question.

Given the population ecology described here the following recommendations can help in conservation of *S. adiaste*. Suitable habitat could be monitored for quality to avoid degradation from human land use including fire suppression and development, or from non-native plants such as grasses that may crowd *Viola purpurea*. Studies of *V. purpurea* populations in grazed and un-grazed areas could help establish whether grazing has positive or negative effects on *S. adiaste* host abundance. Continued monitoring of the Chews Ridge population and others is needed to assess long-term population viability and gain a better understanding of year-to-year fluctuations in population size and how these relate to climate. Habitat with suitable host plants that currently has or has had butterfly colonies should be evaluated for potential habitat reconstruction to create stepping-stones between existing populations. Vacant habitat patches could be identified with the aim of reintroduction in order to improve movement among subpopulations and guard against extinction. Finally, localizing additional population strong holds and documenting patterns of genetic diversity within and between populations can help develop management or recovery plans for this endemic California species.

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