The conservation biology of the Uncompahge fritillary and the related northern dingy fritillary
by Hugh Bryan Britten

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences
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Abstract:
The Boloria improba species group consists of a series of populations of a common biennial arctic butterfly, the northern dingy fritillary (B. improba), which extends from arctic Canada and Alaska down the Rocky Mountain Cordillera with populations in British Columbia, Alberta, and Wyoming. The closely related Uncompahgre fritillary (Boloria acrocnema), known from only two colonies high in the San Juan Mountains of Colorado, occupies the southern end of this distribution. Capture-mark-release (CMR) estimates in 1987 revealed a steep decline in B. acrocnema numbers from earlier estimates at one site and the absence of the butterfly at the other.

CMR and transect count estimates in 1988 indicate that both even-year broods consisted of approximately 250 individuals, a decline of about one order of magnitude from estimates made earlier in the decade.

Eight B. improba group populations were sampled from the Yukon Territory to Colorado and 20 presumptive allozyme loci were assayed from them. Boloria titania were sampled from five B. improba group sites and 18 presumptive loci were assayed from each for comparison. The allozyme data suggest within colony structuring and low levels of gene flow between colonies. Significant negative correlations were found between estimates of allozyme variation and latitude, an indicator of geographical isolation in the B. improba group, that were not found in B. titania. Genetic identity estimates and the geographical distribution of individual alleles suggest that ancestral B. improba existed in Alaskan refugia and south of the major glaciers during the last glacial maximum and that central Canadian B. improba were derived from northern dispersers when the glaciers receded. Results from B. titania concur with this hypothesis.

A principal components analysis of six habitat variables suggests that B. acrocnema may have specialized habitat requirements relative to the rest of the group. A phenetic analysis of 12 wing characters and allozyme results indicate that B. acrocnema is probably a well differentiated subspecies of B. improba rather than a full species. Finally, the results of this study were used to make conservation recommendations for B. acrocnema and to draw some general conclusions about the extinction process.

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Hugh Bryan Britten

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Co-Chairperson, Graduate Committee

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Date 5 June 1991

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ABSTRACT

The *Boloria improba* species group consists of a series of populations of a common biennial arctic butterfly, the northern dingy fritillary (*B. improba*), which extends from arctic Canada and Alaska down the Rocky Mountain Cordillera with populations in British Columbia, Alberta, and Wyoming. The closely related Uncompahgre fritillary (*Boloria acrocnema*), known from only two colonies high in the San Juan Mountains of Colorado, occupies the southern end of this distribution. Capture-mark-release (CMR) estimates in 1987 revealed a steep decline in *B. acrocnema* numbers from earlier estimates at one site and the absence of the butterfly at the other. CMR and transect count estimates in 1988 indicate that both even-year broods consisted of approximately 250 individuals, a decline of about one order of magnitude from estimates made earlier in the decade.

Eight *B. improba* group populations were sampled from the Yukon Territory to Colorado and 20 presumptive allozyme loci were assayed from them. *Boloria titania* were sampled from five *B. improba* group sites and 18 presumptive loci were assayed from each for comparison. The allozyme data suggest within colony structuring and low levels of gene flow between colonies. Significant negative correlations were found between estimates of allozyme variation and latitude, an indicator of geographical isolation in the *B. improba* group, that were not found in *B. titania*. Genetic identity estimates and the geographical distribution of individual alleles suggest that ancestral *B. improba* existed in Alaskan refugia and south of the major glaciers during the last glacial maximum and that central Canadian *B. improba* were derived from northern dispersers when the glaciers receded. Results from *B. titania* concur with this hypothesis.

A principal components analysis of six habitat variables suggests that *B. acrocnema* may have specialized habitat requirements relative to the rest of the group. A phenetic analysis of 12 wing characters and allozyme results indicate that *B. acrocnema* is probably a well differentiated subspecies of *B. improba* rather than a full species. Finally, the results of this study were used to make conservation recommendations for *B. acrocnema* and to draw some general conclusions about the extinction process.

INTRODUCTION

The Uncompahgre fritillary (*Boloria acrocnema*) was discovered on a high alpine meadow near Uncompahgre Peak, Hindsdale Co., CO, in July 1978 (Gall and Sperling, 1980). A second major colony was discovered near Red Cloud Peak, 16 km south of the type locality, in 1982. No other colonies have been located since these initial discoveries; thus *B. acrocnema* is assumed to have the smallest total range of any North American butterfly (Opler, 1990).

Population estimates carried out in 1979 and 1980 at Uncompahgre Peak (Gall, 1984a) and subsequent anecdotal accounts indicated that populations at both *B. acrocnema* locations were declining rapidly. Because of its apparently precarious position, the U.S. Fish and Wildlife Service (USFWS) was petitioned to list *B. acrocnema* under the U.S. Endangered Species Act in 1984. In conjunction with the USFWS, the U.S. Forest Service (USFS), which has jurisdiction over the Uncompahgre Peak *B. acrocnema* site, and the U.S. Bureau of Land Management (BLM), which manages the Red Cloud Peak colony site, began efforts to determine the causes of the butterfly's decline and to formulate a management plan to mitigate their effects. This effort took two years, 1987 and 1988, and resulted in a comprehensive report that addressed the problem of

declining *B. acrocnema* numbers and made several recommendations for the conservation of the butterfly (Brussard and Britten, 1989)

The plight of *B. acrocnema* provides an opportunity to observe a potential natural extinction as it is occurring. Since very little is known about extinction (Ehrlich, 1983), the study of *B. acrocnema* may provide valuable insights into this process.

Current theories about extinction come from population viability analysis (PVA) which is a subfield of conservation biology concerned with predicting the persistence of populations and species. Two general types of processes are recognized as being important in extinctions: 1) deterministic, and 2) stochastic (Brussard, 1986). Deterministic processes have generally predictable outcomes and operate systematically (Brussard, 1986). These types of extinctions are often caused by human activities; for example, the black-footed ferret (*Mustela nigripes*) was eliminated from most of its historic range because its habitat and food requirements were in conflict with the domestic livestock industry.

Stochastic processes are critical to the survival of small populations, and they operate at two levels (Brussard, 1986; Pimm *et al.*, 1988; and Shaffer, 1987). First, population-wide reproduction and survival can be affected by unpredictable environmental change or

catastrophes. Second, random variations in individual survival and reproduction can affect the persistence of very small populations. In addition, population structure and random genetic processes can play roles in the survival of small populations.

Genetic studies of extinction-prone species have mostly been confined to large mammals (e.g. Bonnell and Selander, 1973; Kilpatrick *et al.*, 1986; and O'Brien *et al.*, 1985). The probability of extinction is thought to be increased by the expression of deleterious alleles brought on by inbreeding (inbreeding depression) and the random loss of alleles through drift (e.g. Brussard, 1986; and O'Brien, 1985). Some species, like the cheetah (*Acinomyx jubatus*; O'Brien *et al.*, 1985) and elephant seal (*Mirounga angustirostris*; Bonnell and Selander, 1973), have recovered from major bottlenecks and persist with very little detectable genetic variability. In contrast, other species, such as the black-footed ferret, are evidently very depauperate genetically (although few loci were assayed) and are near extinction in the wild (Kilpatrick *et al.*, 1986). Thus, the exact nature of the interactions between genetic, demographic, and environmental processes are not known but are thought to be important to species survival (Brussard, 1986; Gilpin and Soule', 1986; and Shaffer, 1987). The general consensus appears to be that environmental and demographic uncertainties can pose

immediate threats to the survival of small populations while genetic factors may be more critical to long-term adaptability and persistence (Bonnell and Selander, 1973; O'Brien *et al.*, 1985; and Shaffer, 1987).

Insect population declines and extinctions have been attributed to several factors including habitat loss and fragmentation, unusual climatic conditions, and random genetic processes (Ehrlich, 1983; Pyle *et al.*, 1981; and Thomas, 1983). Although loss of habitat as a result of human activities is considered an important cause of insect (particularly butterfly) extinctions, other activities e.g., pesticide spraying and collecting are not (Ehrlich *et al.*, 1983; Pyle *et al.*, 1981; and Thomas, 1983). Several butterfly distribution changes and extirpations have been attributed to local climatic change (Ehrlich, 1983; Ehrlich *et al.*, 1980; Gilbert and Singer, 1975; Pyle *et al.*, 1981; and Thomas, 1988). Severe drought probably caused population declines and extirpations in two species of *Euphydryas* in California during the mid-1970's (Ehrlich *et al.*, 1980). Drought apparently had a similar effect on many butterfly species in northwestern Europe in 1976 and 1977 (Thomas, 1988). Gilbert and Singer (1975) and Thomas (1988) provide other examples of butterfly extirpations that were probably induced by unusual weather.

The mechanisms by which climate changes reduce populations are probably species-specific and are not

thoroughly understood, but Gilbert and Singer (1975) note that butterfly fecundity, estimated from daily egg production, is reduced in some species during unusually cold weather. In addition, Ehrlich *et al.* (1980) found that a drought disrupted the complex phenological balance between *Euphydryas* ssp. populations and their host plants.

Ehrlich (1983) states two tentative conclusions concerning the genetics of butterfly extinctions. First, inbreeding depression seems to have little effect on butterfly population persistence because once populations are small enough for it to be a factor they usually go extinct for demographic reasons (Ehrlich, 1983). Second, butterfly populations appear to be so precisely genetically adapted to their habitats that artificial reintroductions seldom succeed (Ehrlich, 1983). Thus it appears, as is apparently the case in larger bodied species, that demographic and environmental processes are probably most important to short-term species survival and random genetic processes to long-term persistence.

Gall and Sperling (1980) described *B. acrocnema* as a sister species of a common arctic butterfly, the northern dingy fritillary (*Boloria improba*). Other authors (e.g. Scott, 1986) consider *B. acrocnema* a subspecies of *B. improba*. Although the taxonomic status of *B. acrocnema* has not been resolved, its close relationship to *B. improba* is apparent (Gall and Sperling, 1980; Scott, 1986).

Therefore, the entire *B. improba-B. acrocnema* clade will henceforth be refered to as the "*B. improba* group."

The *B. improba* group in North America consists of a differentiated series of populations that extends from the arctic regions of Canada and Alaska down the Rocky Mountain Cordillera with known populations in British Columbia, Alberta, Wyoming, and Colorado (Figure 1). Several subspecies have been described within this group. Scott (1986) recognizes two subspecies from northern North America; *B. improba improba* from the arctic and *B. improba* ssp. from west-central Alberta. Ferris (1984) describes the disjunct Wyoming subspecies, *B. improa harryi*, from the Wind River Range. Finally, the disjunct *B. acrocnema* is at the southern terminus of the *B. improba* group's distribution in southwestern Colorado (Gall and Sperling, 1980).

Colonies of *B. acrocnema* are found on patches of its larval food plant, snow willow (*Salix nivalis*), on northeast facing slopes at approximately 4100 m elevation. Colony sites are generally mesic as a result of nearby melting snowfields and have about 15% *S. nivalis* ground cover.

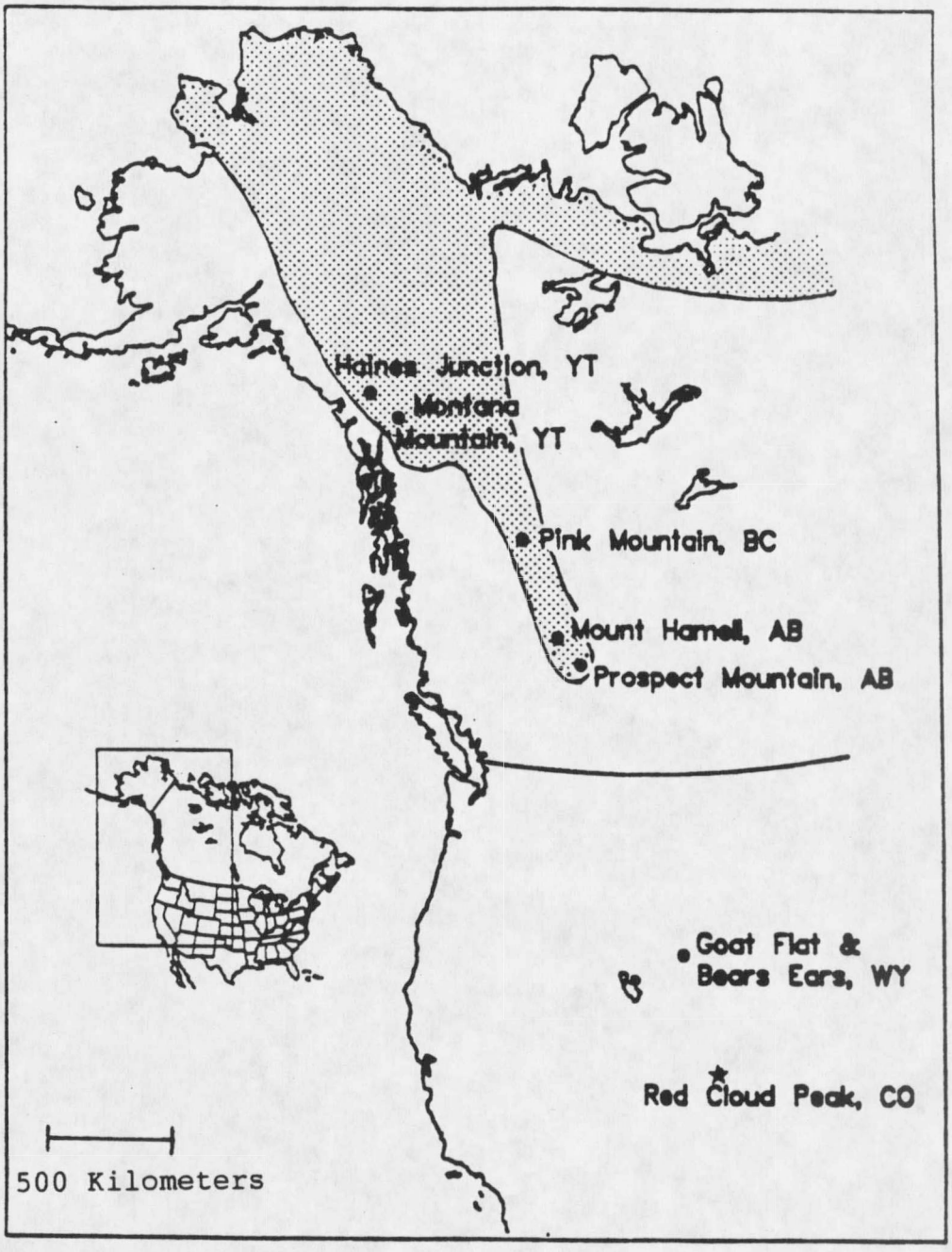

Figure 1. The western North American range of *Boloria improba* and *B. acrocnema* (after Scott, 1986) and sample sites.

A single generation of *B. acrocnema* flies for about 18 days each year starting in mid- to late-July (Scott, 1986). The larvae require two years to develop (Scott, 1986). This results in odd-year and even-year broods which occupy the same sites and may function as essentially independent populations separated in time. *B. acrocnema* adults are extremely sedentary (Gall, 1984a). In a detailed capture-mark-release study Gall (1984a) estimated that mean adult movements rarely exceed 50 m in a flight season. Flight occurs only in full sunlight.

The ecology of *B. improba* is similar to that of *B. acrocnema* (Ferris, 1984; Ferris *et al.*, 1983; and Scott, 1986). Colonies are found at boggy alpine sites that support large areas of *S. nivalis* and *Salix arctica*. Northern populations are found at lower elevations than southern ones. Although all populations in the clade are univoltine, they differ in flight times between regions (Scott, 1986). Canadian *B. improba* begin flying in late June to early July while Wyoming populations fly in late July to early August (pers. obs.; and Scott, 1986). There is no odd-year brood in Alberta (pers. obs.; and Scott, 1986).

The *B. improba* group provides several opportunities for research in the fields of biogeography and conservation biology. Because populations become more geographically isolated to the south (Figure 1), this clade provides a

system to investigate the demography, genetics, and ecology of increasing allopatry. The information gained from the study of these three aspects of *B. improba* group biology may be applicable to conservation efforts aimed at *B. acrocnema*. In order to ascertain the current status of *B. acrocnema*, populations estimates were made at both colony sites in 1987 and 1988. Concurrently, an extensive search for new colonies was undertaken in the San Juan Mountains (Brussard and Britten, 1989). Allozyme analysis was used to address a number of questions pertinent to the conservation of *B. acrocnema*. Genetic comparisons were made among samples from the *B. improba* group in order to assess relative levels of genetic variability, population structure, and the potential genetic effects of geographic isolation. By determining the degree of relatedness among members of this group and by examining the distributions of individual alleles and genetic variability in general, it is possible to form hypotheses concerning the history of the *B. improba* group during the last glacial period. In addition, allozyme data from the *B. improba* group were compared to those from the purple bog fritillary (*Boloria titania*), a much more wide-spread and ubiquitous butterfly whose southern populations are not disjunct in Colorado (Scott, 1986).

Knowledge of the habitat requirements of *B. acrocnema* will be useful in any management decisions that may be made

in the future. By comparing the habitat characteristics of *B. improba* group colonies it may be possible to determine if *B. acrocnema* is a habitat specialist relative to the rest of the group and which colonies are most ecologically similar to one another. This type of information may also be valuable in explaining the decline of *B. acrocnema*. Finally, allozyme and morphometric data (obtained by measuring wing characters) were used to help elucidate the taxonomic status of *B. acrocnema*.

Therefore, although the decline of *B. acrocnema* will probably never be completely understood, knowledge of its demographic, genetic, and ecological characteristics should lead to a more general understanding of similar declines in other species. At the very least, this information will aid in the identification of other species nearing extinction.

STUDY AREAS

Habitat data were collected from 12 occupied *Boloria improba* and *Boloria acrocnema* colony sites distributed from the Yukon Territory to Colorado. Those from which butterfly collections were taken are mapped in Figure 1.

Both Yukon Territory sites are briefly described in Ferris *et al.* (1983). The Haines Junction site is located at 60° 41' latitude 7.5 km S of Haines Junction, YT. It is at 1370 m elevation with an ENE exposure within an extensive area of rolling alpine tundra supporting lush patches of *Salix nivalis* and *Salix arctica* in depressions and moist drainage courses. The Montana Mountain site is located at 60° 09' latitude six km SE of Carcross, YT, on the NNW face of Montana Mountain at 1555 meters elevation. There is standing and running water on this site, and *Salix wolfii* reaches a height of approximately 1.5 meters in parts of it. *S. nivalis* and *S. arctica* are found in areas not shaded by taller shrubs. The site is accesible via mining roads.

The Pink Mountain, BC, site is located on the western aspect of Pink Mountain at 1776 m elevation; 18 km W of Pink Mountain, BC, at 57° 05' latitude. The Pink Mountain colony exists in a long, mesic depression with a lush cover of the larval food plants. This site is near a

fire tower, and there is a dirt road that provides access to it. The Mount Hamell, AB, site is located at 53° 55' latitude 10 km NW of Grande Cache, AB, on a southern exposure of Mount Hamell at 2134 m elevation. The Mount Hamell site is somewhat drier than the others and contains a large fenced exclosure within which there is a dense cover of *S. nivalis*. There are a few krumholtz conifers on the site. The road to the fire look-out tower on the peak of Mount Hamell provides access by foot. The Prospect Mountain, AB, site is located eight km W of Mountain Park, AB, at a latitude of 53° 50'on a NW exposure of Prospect Mountain at 3525 m elevation. This site is on the north side of Prospect Mountain and makes up one side of a steep ravine. *S. nivalis* and *S. arctica* exist in dispersed patches throughout the site. Mining roads provide access by foot.

Both Wyoming *B. improba harryi* colony sites are described in Ferris (1984 and 1986). The Goat Flat site is on a NNW exposure at 3627 m elevation in the Glacier Primitive Area of the Shoshone National Forest located at 43° 19' latitude 24.3 km S of Dubois, WY. It is at the head of a small drainage which contains a snow field. There is a small stream flowing through the site. *S. nivalis* and *S. arctica* are found in a broad band along the edge of the stream. This site is located in one of the most mesic areas of Goat Flat. The Bears Ears site is on

an E exposure at 3475 m elevation in the Popo Agie Primitive Area of the Shoshone National Forest at a latitude of 42° 50' 34 km E of Lander, WY. It lies in a broad draw below Mount Chauvenet. There are intermittent streams fed by melting snow fields up-slope and boggy areas throughout.

B. acrocnema has been located at five sites in the San Juan Mountains of Colorado. Although each of these sites was investigated thoroughly, butterflies were collected from Red Cloud Peak only because of the small numbers found at the other sites. The type locality of *B. acrocnema* is located near Uncompahgre Peak, CO, and is described in Gall and Sperling (1980). The Uncompahgre Peak site has a NE exposure and an elevation of 4100 meters; it is 13.4 km WNW of Lake City, CO, at 38° 07' latitude. A small number of *B. acrocnema* were also discovered at a site 2.4 km SE of the type locality at 3850 meters elevation. This second site has a NNE exposure. Both of these sites are within the Big Blue Wilderness Area of the Uncompahgre National Forest. The Red Cloud Peak, CO, site is located near the top of Red Cloud Peak on a NE exposure at 3890 m elevation 14 km SW of Lake City, CO, at 37° 55' latitude. There are snow fields within and upslope of this site. Red Cloud Peak is similar to the type locality. Both sites have large areas of continuous *S. nivalis* cover and dispersed patches within numerous depressions. The Uncompahgre Peak

site is rockier than the previously described Red Cloud Peak site. A small number of *B. acrocnema* were also discovered at the head of Alpine Gulch which is two km NE of the Red Cloud Peak site. The Alpine Gulch site has an elevation of 3831 meters and a NNE exposure. The Red Cloud Peak and Alpine Gulch sites are administered by the Bureau of Land Management. Finally, a single *B. acrocnema* individual was captured over a gently sloping patch of *S. nivalis* below Baldy Chato 31 km E of Lake City, CO, at 38° 03' latitude in the Uncompahgre National Forest. This site has a NNE exposure and an elevation of 3930 meters.

METHODS

Population Trends

Sizes of *B. acrocnema* populations were estimated at Red Cloud Peak, CO, in 1987 and 1988 and at Uncompahgre Peak, CO, in 1988. *B. improba* colony sizes were estimated at Haines Junction, YT, and Bears Ears, WY, in 1989. The 1987 and 1989 estimates were derived from capture-mark-release (CMR) data used in Lincoln Index (Southwood, 1978) and Jolly-Seber (Gall, 1984b and Southwood, 1978) calculations. In 1988, daily population size estimates were made from CMR data used in Lincoln Index (Southwood, 1978) estimates and transect counts made following the methods of Pollard (1977) and Thomas (1983). In addition, the total numbers of adults that were present in the *B. acrocnema* colonies in 1988 were estimated using Gall's (1984a) estimate of the daily residence rate (the proportion of animals from day i which remain in the population on day i+1), which is 0.60, and the sum of the daily population estimates.

Capture sessions took place on alternate days in 1987 and on consecutive days in 1988 and 1989. CMR field work was done during the daily peak flight period (i.e., 0900 h to 1400 h) under favorable flight conditions. Two to four

people participated in capturing butterflies during each CMR capture run. Butterflies were netted and placed, with wings folded, in glassine envelopes. The envelopes were kept shaded and dry until the captured butterflies were processed. Capture efforts were suspended to allow shading clouds to pass over the site. Capture periods lasted from one to three hours and were terminated when no butterflies were captured for 15 to 20 minutes.

After a capture run was completed butterflies were marked with individually coded numbers which were placed on the ventral surfaces of the wings with an indelible ink marker. The position of each mark corresponded to a digit in the unique number given to each butterfly (Brussard, 1970). Three categories of wing wear were recorded: 1) fresh- unfaded colors, no loss of scales, and no tearing; 2) intermediate- colors faded, some loss of scales, no tearing; 3) worn- colors faded, loss of scales, wing margins torn. The sex of each butterfly was noted by examination of the posterior end of the abdomen for valvae in males and ovipositors in females. Finally, the event (capture, recapture, or sacrifice) was recorded for each butterfly. Animals to be released for subsequent recapture attempts were placed on unshaded vegetation within the colony site and left undisturbed. The released butterflies usually basked for 10 to 15 minutes until high enough body temperatures were attained to allow flight. Some male

butterflies were sacrificed during CMR studies for inclusion in the electrophoretic analyses.

B. acrocnema population trends were monitored throughout the 1988 flight season at Uncompahgre Peak, CO, and Red Cloud Peak, CO, using transect counts (Pollard, 1977 and Thomas, 1983). Transect counts provide relative estimates of population size while minimizing disturbances to the butterflies. Transects were established at the two sites as early in the 1988 flight season as possible. Each transect was 100 meters long and was placed along topographic contours within each site through areas of butterfly concentration. An observer walked at a steady pace (8-10 minutes/transect) and counted all *B. acrocnema* that flew within five meters of the transect. Counts were begun between 0900 h and 0930 h each day, and the same observer was used for each site throughout the flight season. The observer only walked during periods of good flight conditions (i.e., full sun and minimal winds) and would wait until clouds passed overhead or winds subsided. Counting could be stopped to allow the observer to rest or to follow a butterfly for a positive identification, but counting then resumed at the point where it was stopped.

Their size, weak and low flight, and the dark area near the base of the wings make *B. acrocnema* identifiable by trained personnel at considerable distances. *Euphydryas editha* may be mistaken for *B. acrocnema* at the beginning of

the flight season, but *E. editha* flies more strongly and higher than *B. acrocnema*. Furthermore, its numbers decline greatly as the *B. acrocnema* flight season progresses.

In order to estimate daily population numbers from the transect counts taken throughout the the 1988 flight season a CMR study was carried out late in the flight season at Red Cloud Peak, CO. The Lincoln Index and Jolly-Seber population estimates derived from the CMR study were then used to calibrate the daily transect counts from this colony. Transect counts for each day of the 1988 CMR study were divided by the population estimate to yield an estimate of the percentage of the population seen during the transect walk on that day. This percentage was then used to extrapolate daily population estimates from all the transect counts done during the flight season. It was assumed that the percentage of butterflies encountered on transect walks was constant throughout the flight season. Likewise, daily population estimates at Uncompahgre Peak, CO, were extrapolated from transect counts and the estimated percentage of counted butterflies at Red Cloud Peak, CO. It was assumed that the same percentage of the butterfly population counted during transect walks at Red Cloud Peak, CO, was counted at Uncompahgre Peak, CO.

Population Genetics

B. acrocnema were collected from the Red Cloud Peak site in 1987 and 1988. Only males were taken late in the flight season to minimize the effects of collecting on the demography of the population. *B. improba* were collected from seven sites in Canada and Wyoming in 1989 and 1990 (Figure 1). Samples for between-year comparisons were obtained at the Bears Ears, WY, and the Red Cloud Peak, CO, sites. Sample sizes are given in Table 1. In addition, *Boloria titania* were collected from three of the Canadian *B. improba* sites and at both the *B. acrocnema* sites in Colorado to provide a basis of comparison with the *B. improba-B. acrocnema* clade. Sizes of *B. titania* collections are given in Table 2.

Collections were made during favorable flight conditions (i.e., full sunlight, light winds, and 16-20^{o}C temperatures). These conditions occured most predictably between 0900 h and 1400 h each day. Butterflies were collected and placed into glassine envelopes; after collection the wings were removed and the bodies were placed in ampules in liquid nitrogen which killed by freezing. Specimens were stored in an ultra-cold freezer at -80^{o}C until they were prepared for electrophoretic analysis.

Table 1. Sample sizes and alleles frequencies at polymorphic allozyme loci in eight *B. improba* group populations. Sample sites are: A--Haines Junction, YT, B--Montana Mountain, YT, C--Pink Mountain, BC, D--Mount Hamell, AB, E--Prospect Mountain, AB, F--Bears Ears, WY, G--Goat Flat, WY, and H--Red Cloud Peak, CO.

Region	North		Central			South		
Colony	A	B	C	D	E	F	G	H
N	17	50	47	22	17	38	28	34
AGP								
C	.588	.400	.926	.341	1.000	.000	.107	.000
D	.412	.600	.074	.659	.000	1.000	.893	.588
E	.000	.000	.000	.000	.000	.000	.000	.412
IDH-1								
C	.912	.980	1.000	1.000	1.000	1.000	1.000	1.000
D	.088	.020	.000	.000	.000	.000	.000	.000
IDH-2								
B	.000	.052	.000	.000	.000	.000	.000	.000
C	1.000	.948	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH								
C	1.000	1.000	.989	1.000	1.000	1.000	1.000	1.000
D	.000	.000	.011	.000	.000	.000	.000	.000
AAT-1								
B	.000	.031	.000	.000	.000	.000	.000	.000
C	.733	.573	.223	.000	.000	1.000	1.000	1.000
D	.233	.323	.585	1.000	1.000	.000	.000	.000
E	.033	.073	.191	.000	.000	.000	.000	.000
AAT-2								
D	.971	.980	.567	1.000	1.000	1.000	1.000	1.000
E	.029	.020	.433	.000	.000	.000	.000	.000
HBDH								
A	.971	1.000	.989	1.000	1.000	1.000	1.000	1.000
B	.029	.000	.011	.000	.000	.000	.000	.000
MPI								
D	.000	.000	.000	.000	.206	.000	.000	.000
E	.059	.020	.000	.477	.471	.000	.000	.000
F	.941	.980	1.000	.523	.324	1.000	1.000	1.000

Table 1, Continued.

SOD								
C	1.000	.950	1.000	1.000	1.000	1.000	1.000	1.000
D	.000	.050	.000	.000	.000	.000	.000	.000
PGM								
E	.059	.000	.078	.523	.912	.000	.000	.000
F	.941	1.000	.922	.477	.088	1.000	1.000	1.000
G6PDH								
C	.000	.410	.266	.000	.000	.000	.000	.000
D	1.000	.590	.734	1.000	1.000	1.000	1.000	1.000
PGD								
C	.029	.050	.000	.000	.000	.000	.000	.000
D	.971	.790	1.000	1.000	1.000	1.000	1.000	1.000
E	.000	.160	.000	.000	.000	.000	.000	.000
LDH								
B	1.000	1.000	1.000	1.000	1.000	.987	1.000	1.000
C	.000	.000	.000	.000	.000	.013	.000	.000
GPI								
A	.000	.000	.000	.000	.000	.000	.000	.074
B	.029	.130	.000	.000	.000	.000	.000	.926
C	.824	.830	1.000	1.000	1.000	1.000	.982	.000
D	.147	.040	.000	.000	.000	.000	.018	.000
MDH-1								
B	1.000	1.000	1.000	1.000	1.000	1.000	.982	1.000
C	.000	.000	.000	.000	.000	.000	.018	.000
MDH-2								
B	.000	.020	.000	.000	.000	.000	.000	.000
C	.971	.980	.596	.682	1.000	1.000	1.000	1.000
D	.029	.000	.404	.318	.000	.000	.000	.000

Specimens were prepared for electrophoresis by crushing the whole body in a centrifuge tube with 0.2 ml of cold buffer (0.05 M tris, pH 7.1). They were then centrifuged at 12,000 rpm for five minutes. The supernatant was applied to 14% starch gels using a filter paper wick.

Table 2. Sample sizes and allele frequencies at polymorphic allozyme loci in five *B. titania* populations. Sample sites are: A--Haines Junction, YT, B--Pink Mountain, BC, C--Prospect Mountain, AB, D--Uncompahgre Peak, CO, and E--Red Cloud Peak, CO.

Region	North	Central		South	
Colony	A	B	C	D	E
N	59	12	14	69	54
AGP					
B	.000	.000	.000	.009	.000
C	1.000	1.000	1.000	.982	.981
D	.000	.000	.000	.009	.019
IDH-1					
B	.578	.083	.000	.016	.019
C	.422	.917	1.000	.918	.935
D	.000	.000	.000	.066	.037
IDH-2					
A	.000	.042	.000	.000	.000
B	.000	.875	.000	.008	.009
C	1.000	.083	1.000	.975	.982
D	.000	.000	.000	.017	.000
E	.000	.000	.000	.000	.009
GAPDH					
C	1.000	1.000	1.000	.943	.945
D	.000	.000	.000	.057	.055
AAT-1					
B	.000	.000	.000	.000	.009
C	.974	.917	1.000	1.000	.973
D	.026	.083	.000	.000	.018
AAT-2					
B	.000	.917	.000	.008	.009
C	1.000	.083	.964	.992	.964
D	.000	.000	.036	.000	.027
PEP-GL					
C	.992	1.000	1.000	1.000	1.000
D	.008	.000	.000	.000	.000

Table 2, Continued.

MPI					
B	.228	.000	.036	.000	.018
C	.439	.083	.929	.968	.927
D	.325	.083	.036	.032	.055
E	.009	.375	.000	.000	.000
F	.000	.458	.000	.000	.000
SOD					
A	.102	.000	.000	.000	.000
B	.000	.000	.000	.032	.009
C	.898	1.000	1.000	.929	.955
D	.000	.000	.000	.040	.036
PGM					
A	.081	.000	.000	.000	.000
B	.486	.083	.000	.036	.033
C	.027	.500	.583	.580	.587
D	.081	.250	.417	.377	.370
E	.000	.167	.000	.007	.011
F	.324	.000	.000	.000	.000
G6PDH					
B	.021	.000	.000	.000	.000
C	.021	.042	.000	.710	.648
D	.958	.958	1.000	.290	.352
PGD					
A	.039	.000	.000	.000	.000
B	.579	.083	.050	.000	.000
C	.053	.000	.000	.710	.855
D	.316	.042	.950	.290	.145
E	.000	.292	.000	.000	.000
F	.013	.583	.000	.000	.000
GPI					
B	.092	.000	.000	.008	.000
C	.816	.083	.000	.048	.064
D	.066	.083	1.000	.379	.636
E	.013	.833	.000	.290	.227
F	.013	.000	.000	.048	.064
G	.000	.000	.000	.000	.009
MDH-1					
B	.908	.375	.000	.016	.000
C	.092	.625	.893	.968	1.000
D	.000	.000	.107	.016	.000

Table 2, Continued.

MDH-2					
A	.474	.292	.000	.000	.000
B	.171	.667	.000	.000	.000
C	.342	.042	1.000	.984	.991
D	.013	.000	.000	.016	.009

Allozyme variation was assayed at 20 presumptive loci in *B. improba* and *B. acrocnema* and at 18 presumptive loci in *B. titania* (Table 3). General methods and staining procedures were similar to those described by May *et al.* (1979). Genotype frequencies were obtained by direct count from the phenotypes observed on the gels, and inferred electromorph (allozyme) frequencies were calculated from the genotype frequencies.

Expected genotypic frequencies, fixation indices (F-statistics), and fit to Hardy-Weinberg expectations were calculated using BIOSYS-1 (Swofford and Selander, 1981). Heterozygote deviation (D) from Hardy-Weinberg expectations was calculated for each population at each locus. This statistic indicates whether the observed numbers of heterozygotes at each locus are in excess, deficient or identical to the expected under Hardy-Weinberg conditions. A Sign Test (Daniels, 1990) was used to test for significant patterns of heterozygote deviation within samples.

In order to elucidate further the nature of any population substructure within sampled *B. improba* group colonies, the inbreeding coefficient (F) was estimated across all polymorphic loci in each sampled colony (Hartl, 1980). Mean F was then calculated using the eight within colony inbreeding coefficients obtained by this procedure. In addition, overall conformance with Hardy-Weinberg expectations was tested across all *B. improba* group samples by adding independently derived expected numbers of heterozygotes from all samples to yield a total expected number of heterozygotes under Hardy-Weinberg assumptions. This sum was then tested against the total observed number of heterozygotes across all samples in a X^2-test. A X^2-test for heterogeneity (Sokal and Rohlf, 1981) was used to test for allele frequency differences between samples at each locus. This test, like F_{st}, gives an indication of among subpopulation variance in allele frequencies at each locus. The effective number of migrants per generation (Nm) was estimated from the F-statistics following Slatkin (1987). Nei's (1978) unbiased genetic identity (I) was used to calculate overall genetic similarity among populations. UPGMA (Sokal and Sneath, 1973) was used to cluster genetic identities to derive phenograms of similarity among populations. Correlation coefficients between latitude and mean individual heterozygosity, percent polymorphic loci, and mean number of alleles per

locus were calculated using Spearman's rank correlation (Sokal and Rohlf, 1981). A qualitative analysis of geographic allelic distributions was also done to elucidate patterns of allelic distributions among regions. Regions were delineated as: 1)North-- Territory sites, 2) Central-- the three sites in British Columbia and Alberta, and 3) South-- the three sites in Wyoming and Colorado.

Table 3. Loci and electrophoretic conditions used to assay *B. improba* group and *B. titania* populations.

Locus	Enzyme	Buffer
AGP	Alpha-glyerophosphatase	C*
AAT-1,2	Aspartate aminotransferase	C
DIA	NADH diaphorase	4**
GP	General protein	R***
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	C
GPI	Glucosephosphate isomerase	4
G6PDH	Glucose-6-phosphate dehydrogenase	4
@ HBDH	Hydroxybuteric dehyrogenase	R
IDH-1,2	Isocitrate dehydrogenase	C
@ LDH	Lactate dehydrogenase	4
MDH-1,2	Malate dehydrogenase	C
MPI	Mannosephosphate isomerase	R
PEP-GL	Peptidase (glycyl-leucine)	R
PGD	Phosphogluconate dehydrogenase	4
PGM	Phosphoglucomutase	4
SOD	Superoxide dismutase	R
XDH	Xanthine dehydrogenase	R

* Electrode buffer: 0.04 mol/dm^3 citric acid adjusted to pH 6.1 with N-(3-amino propyl)-morpholine; diluted 1:10 for gel buffer (Clayton and Tretiak, 1972).
** Selander et al. (1971).
*** Ridgeway et al. (1970).
@ Assayed in *B. improba* only.

Habitat Analysis

A set of 21 habitat variables was measured at each of the seven *B. improba* colony sites from which butterflies were collected (Figure 1). The same set of variables was measured at all five sites at which one or more *B. acrocnema* were captured. Upper and lower elevations were measured with an altimeter calibrated at a locality with known elevation each morning and corroborated with topographic maps. Mean elevations were then calculated for each site. Mean elevations were standardized to the latitude of the Red Cloud Peak, CO, site to remove latitudinal differences among sites in subsequent comparisons (MacArthur, 1972). One hundred meters were added to the elevation of each site that is north of Red Cloud Peak for every degree of latitudinal difference between the site and Red Cloud Peak, CO. Percent slope was measured with a clinometer and checked using a land area slope indicator for topographic maps (Reproductions Specialties, Inc., Denver, CO 80222). Aspect was measured in the field with a compass and verified on topographic maps. It was indexed on a relative scale based on Wentworth (1976) with the most mesic (NNE facing) sites being assigned ones and the most xeric (SSW facing) sites being scored as nines. Site length was measured

to the nearest five meters using a tape measure. Site edge characteristics were quantified by walking the perimeter of each site and estimating the percentage of willow, meadow, talus, scree, rock face, and snow edge components. The percentage of each cover component was estimated for each site using a line intercept method (Smith, 1980). Three 50-meter transects were randomly placed at each site, and a tape measure was used to measure cover components. Sites that were too small to accommodate three 50-meter transects were measured with fewer and shorter transects according to their size. Cover components measured were: larval food plants (*Salix nivalis* for *B. acrocnema* and *S. nivalis* and *Salix arctica* combined for *B. improba*), non-food shrubs (e.g. *Salix wolfii*, *Dryas hookeriana*, and *Arctostaphylos uva-ursi*), forbs, grass, moss, lichen, bare soil, litter, small rocks (diameter<15 cm), and large rocks (diameter>15 cm). Percent cover for each cover component was calculated by dividing the total length of each component within a transect by the total length of the transect and then averaging for each cover component over all transects measured at each site. Frequency of nectar sources was estimated by tallying by species all flowers in bloom that touched the tape measure during the line intercept measurements. The counts for each species on which *B. acrocnema* (*Aster sp.*, *Gentiana prostrata*, *Hymenoxys sp.*, and *Silene acaulis*) or *B. improba* (*Cassiope sp.*, *Caltha*

canadensis, *Myostis alpestris*, and *Silene acaulis*) was observed feeding were added together, and this total was divided by the total number of flowers in bloom touching the tape measure. Flower counts for all transects measured on a site were pooled for this estimate.

Sites were grouped into four regions for preliminary statistical analyses. The regions were: 1) Yukon Territory, 2) British Columbia and Alberta, 3) Wyoming, and 4) Colorado. A single classification analysis of variance (Sokal and Rohlf, 1981) among regions was carried out for each habitat variable to identify variables that differ significantly among regions. Once identified by the ANOVA, these variables were used in a principal components analysis to determine the pattern of similarity among sites with respect to the habitat variables that differed among regions.

Phenetic Measurements

Twenty-five *B. improba* group individuals were randomly selected for inclusion in the phenetic analysis. Numbers of individuals used from each collection site are: 1) six *B. improba improba* from Montana Mountain, YT, 2) one *B. i. improba* from Pink Mountain, BC, 3) three *B. i. youngi* from Mount Hamell, AB, 4) three *B. i. youngi* from Prospect Mountain, AL, 5) three *B. i. harryi* from Bears Ears, WY, 6) three *B. i. harryi* from Goat Flat, WY, and 7) six *B.*

acrocnema from Red Cloud Peak, CO. Subspecies designations follow Gall and Sperling (1980). In addition, *B. bellona* specimens from a variety of localities were included in the analysis as an outgroup because they are morphologically distinct from the *B. improba* group. They were obtained from the entomology museum at Montana State University.

A binocular dissecting microscope equiped with an ocular micrometer was used to measure eight continuous characters and four discrete characters on the right wing of each butterfly (Table 4). Six *B. improba* group individuals were selected at random and three replicate sets of measurements were taken from them. Standard errors were calculated for each individual at each character. The mean standard error for each character was calculated to provide an estimate of measurement error. *B. acrocnema* voucher specimens are stored at the entomology museum at Montana State University.

Principal components analysis based on the correlation matrix of characters was used to cluster the individuals graphically. The first two principal components were used to derive scatter plots of the position of each individual on principal component axes one (PC1) and two (PC2). Euclidean distances were used to measure the separation between clusters. Two PCA's were performed; one that included *B.i. harryi* and one that did not.

Table 4. Wing characters measured from *B. bellona* and *B. improba* group individuals.

Character	Type*
Dorsal Surface	
1. FW length	C
2. FW ground color	D
3. Extent of white on FW	D
4. Width of FW postmedian spot in cell M_3	C
5. Width of FW postmedian spot in cell R_5	C
6. Distance from FW postmedian spot in cell R_4 to costal margin	C
7. Distance from FW postmedian spot in cell M_2 to outer margin	C
8. Width of FW postmedian band in cell M_3	
9. Extent of melanization on HW	D
Ventral Surface	
10. Length of HW postmedian-median discal spot	C
11. Distance from HW postmedian-median discal spot to outer margin	C
12. Extent of purple on HW	D

* C=continuous character; D=discrete character scored 1-4.

RESULTS

Population Trends

1987 Population Estimates

On 18 July 1987 two butterflies were tentatively identified as *B. acrocnema* at the Red Cloud peak, CO, site. A flight was found to be in progress at the site on 24 July. Sixty-seven individuals were captured that day. Of these, 63 were marked and released, and four (three males and one female which were damaged by handling) were frozen for later electrophoretic analysis. The site was visited two days later, and 38 individuals were captured, 19 of which were recaptures. All of these were marked and released. On 28 July only 17 individuals were found, six of which were recaptures. Since the population was obviously declining and it was unlikely that many females remaining in the population would be unmated, the decision was made to retain all seven males captured for electrophoresis. On 30 July, 13 individuals, including one female recapture, were taken; nine more males were frozen. On 1 and 3 August, seven and two individuals, respectively, were captured and released.

Overall recapture frequencies did not differ significantly between males and females (G-test, $p>0.100$);

therefore, both data sets were combined for analysis. Based on the pooled data, Lincoln Index estimates and their standard deviations (Southwood, 1978) for 26 and 28 July 1987 were 126 ± 20 and 129 ± 49 respectively. The Jolly-Seber estimate (Southwood, 1978 and Gall, 1984b) for 26 July 1987 was 141.

These estimates are not robust for several reasons. Because the population appeared to be declining during the sampling period the Lincoln Index was technically inappropriate, and since the size of the recapture sample was not predetermined, the estimates of the standard deviations were inappropriate as well (Southwood, 1978). The Jolly-Seber technique was appropriate for this data set (Southwood, 1978); however, the small sample sizes and the paucity of recaptures past 27 July precluded estimating population size other than for 26 July or for even calculating the variance of this estimate. Because of these limitations, the above estimates of daily population size must be viewed with a great deal of caution.

Nevertheless, it is highly desirable to know what the size of the odd-year brood was at Red Cloud Peak, CO, in 1987. Figure 2 shows the number of individuals handled each day of the CMR, the minimum possible population size for those days. The capture sex ratios did not differ from unity at any time, nor did the proportion of fresh individuals differ between males and females for 24 July

(Mean=0.58), 26 July (mean=0.35), or the period from 28 July to 3 August (mean=0.35 [Table 5]).

Judging from the data shown in Figure 2 and from the proportion of fresh individuals in the daily samples (Table 5), the population was probably slightly past its peak emergence on 24 July (a reasonable assumption since the first individuals were seen on 18 July). In a detailed study of the population at Uncompahgre Peak, CO, in 1980 Gall (1984a) sampled on approximately the same dates as this study. Gall (1984a) concluded that the first part of the flight season had been missed in that study as well. The two studies had fairly comparable mean number of captures per individual (1.6 in Gall, 1984a, and 1.3 in the present study), and the areal extent of the two habitats is about equal. Thus, both studies appear to represent reasonably equivalent sampling efforts. If so, there should be an equivalent ratio of the number of butterflies handled to total population size. Gall (1984a) captured about 2.5 times as many butterflies as were captured for the present study (359 vs. 144), so, following this line of reasoning, the total population size at Uncompahgre Peak, CO, in 1980 (650-750) should have been approximately 2.5 times larger than that at Red Cloud Peak, CO, in 1987. This results in an estimate of 250-300 individuals at Red Cloud Peak, CO, in 1987.

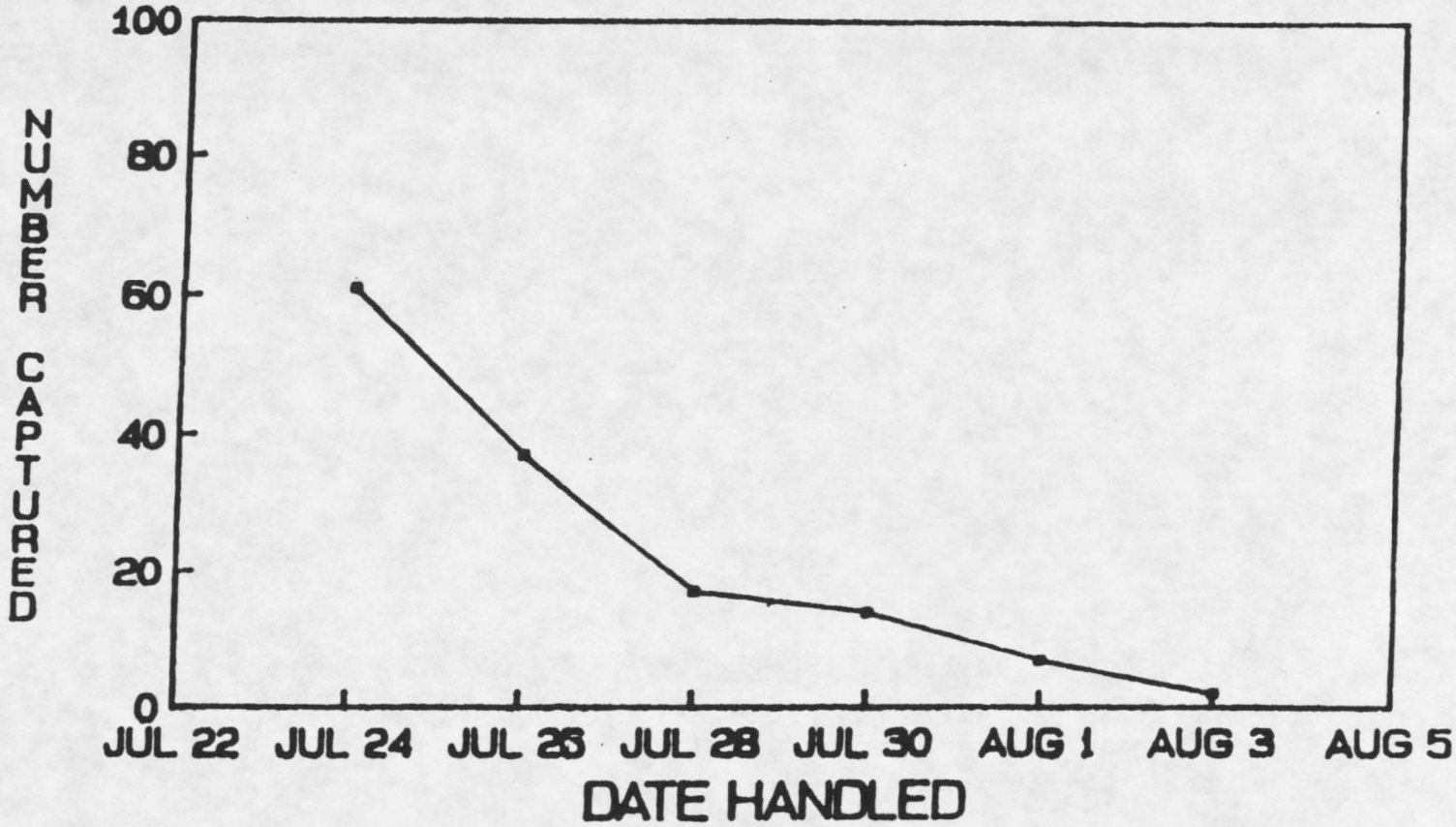

Figure 2. Number of *B. acrocnema* handled each day of the 1987 capture-mark-release (CMR) study at Red Cloud Peak, CO.

There was no flight of *B. acrocnema* at its type locality (Uncompahgre Peak, CO) in 1987 although three insects tentatively identified as this species were seen (but not captured) on 16 July and 20 July. The site was visited again on 22 July, but a thorough search of the area failed to locate any *B. acrocnema*. The site was visited four additional times during late July and early August, but no individuals were encountered.

Table 5. Sex ratio and proportion of fresh *B. acrocnema* captured at Red Cloud Peak, CO in 1987.

	Capture Sex Ratio		Proportion Fresh	
Date	Males	Females	Males	Females
24 July	28	39	.59	.57
26 July	18	20	.29	.40
28 July	8	9	.33	.25
30 July	9	4	.56	.25
1 August	5	2	.40	0
3 August	1	1	0	0

Two fresh male *B. acrocnema* were collected at the second Uncompahgre Peak, CO, site (2.4 km SW of the type locality) on 22 July, and one fresh female and one intermediate male were marked on 27 July. The site was subsequently visited twice, but no additional *B. acrocnema* were found.

1988 Population Estimates

The first *B. acrocnema* of 1988 were seen at Red Cloud Peak, CO, on 10 July, eight days earlier than in 1987. Six males and four females were captured and released, and about 15 others were seen in flight on 10 July. Six 100-meter transects were placed in areas of butterfly concentration at Red Cloud Peak, CO, on 11 July, and the first transect counts were taken. A seventh transect was established on 20 July in the northwest corner of the Red Cloud Peak, CO, site. The last transect count was made on 23 July. Figure 3 gives the daily counts throughout the flight season.

A CMR study was carried out between 22 July and 28 July, 1988 at Red Cloud Peak, CO. A total of 52 butterflies, including six recaptures, were handled (Figure 3). Sexes were combined for analysis although five of the six recaptures were females. Table 6 presents the capture sex ratios and proportion of fresh individuals captured for each sex. It is apparent from the excess of females and the lack of fresh individuals late in the sample period that the population was declining during the CMR. For this reason, along with the very small number of recaptures

during the last four days of the CMR, these days were not included in making the population estimates.

Although technically inappropriate because inflated estimates result when sampled populations are declining, a Lincoln Index estimate was made for 22 July, and a Bailey Triple Catch estimate (a modified Lincoln Index that uses data from three days [Southwood, 1978]) was made for 23 July 1988. The estimated population sizes were 100 for 22 July and 12 for 23 July (Figure 3). The ratio of transect count to population estimate for 22 July was 0.090 and for 23 July this ratio was 0.085. Therefore, the transect counts may have accounted for about 10 percent of the population. If this ratio held throughout the flight season, it is possible to extrapolate the population size for all days on which transect counts were taken using this figure. These estimates are shown in Figure 3; the same caution used when considering the 1987 population estimates should be used for the 1988 estimates.

There was an estimated total adult *B. acrocnema* population size of 492 at Red Cloud Peak, CO, in 1988. Based on the apparent low density of *B. acrocnema* on any probably an overestimate of the total of adult butterflies present at the site in 1988.

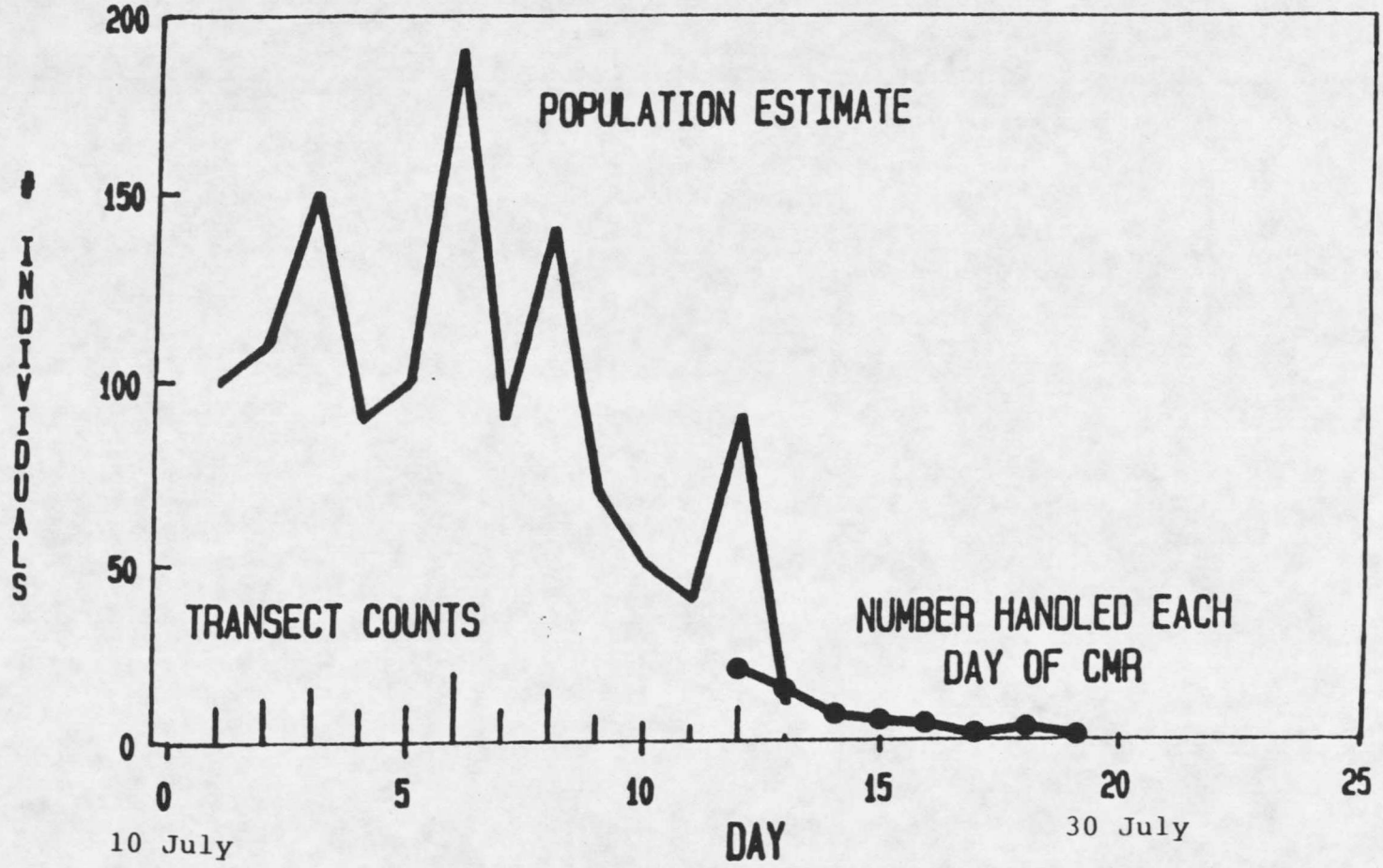

Figure 3. Transect counts, numbers handled each day of capture-mark-release (CMR) study, and population size estimates of *B. acrocnema* at Red Cloud Peak, CO during the 1988 flight season.

Table 6. Sex ratio and proportion of fresh *B. acrocnema* captured at Red Cloud Peak, CO in 1988.

	Capture Sex Ratio		Proportion Fresh	
Date	Males	Females	Males	Females
22 July	10	10	.10	.10
23 July	5	7	0	0
24 July	2	2	0	0
25 July	1	4	0	0
26 July	0	2	0	0
27 July	0	0	0	0
28 July	0	3	0	0

B. acrocnema was present at the type locality, Uncompahgre Peak, CO, in 1988. Five 100-meter transects were established on this site on 12 July, and transect counts were begun. Counts continued at Uncompahgre Peak through 27 July, and the results are shown in Figure 4. Six *B. acrocnema* were also seen at two nearby sites on 19 and 21 July. Since the *S. nivalis* is continuous between these sites this probably did not represent the discovery of new colonies. If it is assumed that the same percentage of the butterfly population counted during transect walks at Red Cloud Peak, CO, (about 10%) was counted at Uncompahgre Peak, CO, population estimates can be made from the transect counts for each day (Figure 4). The total number of adult butterflies present at the Uncompahgre Peak, CO, site during the 1988 flight season was approximately 208.

Three 100-meter and one 70-meter transects were established at the second Uncompahgre Peak *B. acrocnema* site on 12 July and the transects were walked through 22 July. Only two butterflies were seen at this site, one each on 12 July and 14 July.

Two new *B. acrocnema* locations were discovered in 1988. Three *B. acrocnema* (one male and two females) were captured on the upslope portion of Alpine Gulch (2.0 km NE of the Red Cloud Peak, CO, site) on 19 and 20 July. This site was searched in 1987 and was found to be unoccupied. One female *B. acrocnema* was captured near Baldy Chato on 27 July 1988. Two others were seen in flight at that time. This is noteworthy because of the relatively long distance (about 56 km) between the Baldy Chato site and the previously known occupied sites.

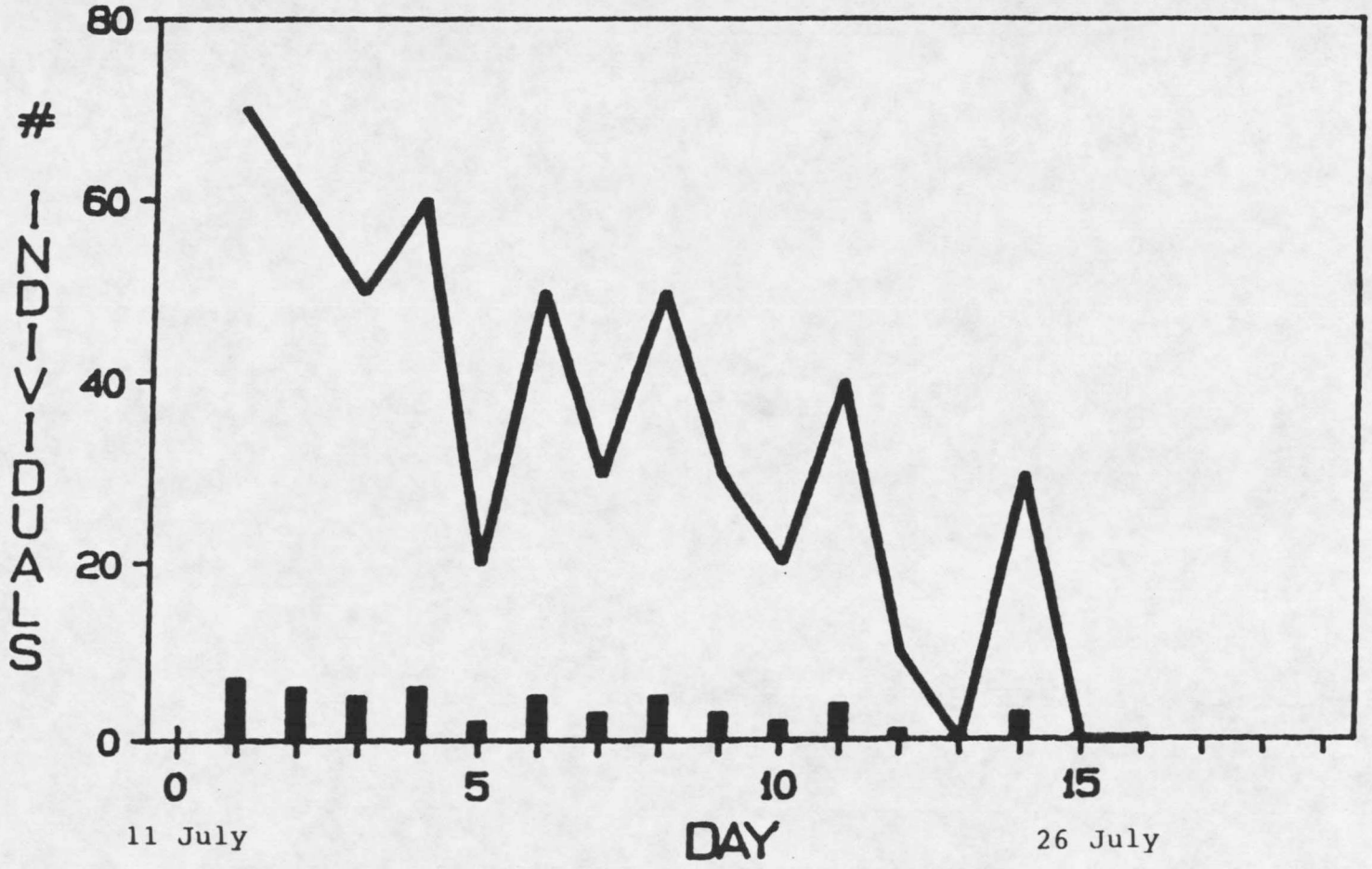

Figure 4. Transect counts (bars) and population size estimates (line) of *B. acrocnema* at Uncompahgre Peak, CO during the 1988 flight season.

1989 Population Estimates

Five (four females and one male) *B. improba* were captured and marked as part of a CMR study at the Haines Junction, YT, site on 24 June 1989. All the captured individuals were fresh. A recapture run was attempted on 25 June but poor weather conditions made field work impossible. Ten males and one female *B. improba* were captured at Haines Junction, YT, on 26 June. Three of the males had worn wings and the rest of the sample had fresh wings. There were two recaptured males in the 26 June sample, but these butterflies were recaptured on the ground in the same place where they were released two days before. Some Lincoln Index assumptions had apparently been violated (Southwood, 1978); the two recaptured individuals probably did not mix back into the population, and handling probably affected their post-marking behavior. Given these reservations, the Lincoln Index population size estimate for the Haines Junction, YT, *B. improba* colony on 24 June 1989 was 28 ± 16. All 11 individuals captured on 26 June were frozen for electrophoresis.

Nine male and seven female *B. improba harryi* were captured on 28 July 1989; the first day of a CMR study at Goat Flat, WY. Eleven percent of the males and 29% of the females in this sample were fresh. All were marked and

released. A total of 28 butterflies was captured at the same site on 29 July; 16 of these were males and 12 were females. There were two female and one male recaptures in this sample. The 29 July sample contained 13% fresh males and no fresh females. Sexes were combined for statistical analysis. The Lincoln Index population estimate for the Goat Flat, WY, *B. improba harryi* colony on 28 July 1989 was 149 ± 61 butterflies. All individuals captured on the second day of the CMR were frozen for electrophoresis.

Population Genetics

Boloria improba Group

Table 1 contains the inferred allelic frequencies in the *B. improba* and *B. acrocnema* populations as sampled. There were no significant differences in allelic frequencies between odd-year and even-year samples for *B. acrocnema* at Red Cloud Peak, CO. Nei's (1972) genetic distance between the two years was 0.0002 over all 20 loci. Very low F_{st} values at the two polymorphic loci, GPI and AGP (Fst=0.0008 and 0.003 respectively), also indicated that between year differences among samples at Red Cloud Peak were insignificant. Similarly, allelic frequencies between the 1989 and 1990 *B. improba harryi* samples from Bears Ears, WY, were statistically identical. Both broods were fixed for the same alleles at all loci except for the presence of one heterzygous individual at the LDH locus in

the 1989 sample. Since sample sizes were inadaquate to make valid between year comparisons at the other *B. improba* group sites, it was assumed that these populations also had small allelic frequency differences between years. So assuming, samples of alleles were pooled across years from sites at which samples from more than one year were obtained.

There were some indications that *B. improba* group colonies are microgeographically structured with respect to gene flow. Six of 20 loci (AGP, AAT-1,2, G6PDH, PGD, and MDH-2) were not in Hardy-Weinberg equilibrium in the Montana Mountain, YT, sample and three of 20 loci (AAT-1, PGM, and G6PDH) did not conform to Hardy-Weinberg expectations in the Pink Mountain, BC, sample. The Haines Junction, YT, and Red Cloud Peak, CO, samples each had one of 20 loci (AGP and GPI, respectively) out of Hardy-Weinberg equilibrium. All significant deviations from Hardy-Weinberg expectations were caused by heterozygote deficiencies. All other loci were in Hardy-Weinberg equilibrium in the remaining samples. The Montana Mountain, YT, site had a significant pattern of heterozygote deficiency (nine of 11 polymorphic loci were heterozygote deficient; $p=0.0328$, Sign Test) as did the Mount Hamell, AB, (all four polymorphic loci were heterozygote deficient) and Red Cloud Peak, CO, (both polymorphic loci were heterozygote deficient) samples.

Additionally, there was a significant deficiency of heterozygotes over all *B. improba* group samples ($p<0.005$). Small-scale structuring within colonies was also indicated by a high estimate of the mean within colony inbreeding coefficient ($F=0.159$). In comparison, population-wide matings between half-siblings over one generation would yield an inbreeding coefficient of $F=0.125$ (Hartl, 1980).

Genetic variability was estimated within each sample with three measures: 1) mean population heterozygosity, 2) percentage of polmorphic loci, and 3) mean number of alleles per locus. These estimates are in Table 7. There was a statistically significant positive correlation between percentage of polymorphic loci and latitude ($r=0.8916$, $p=0.0183$). Similar positive correlations were found between the mean number of alleles per locus and latitude ($r=0.9398$, $p=0.0129$), and between mean expected heterozygosity and latitude ($r=0.8813$, $p=0.0038$). Populations of the *B. improba* group did seem to become more isolated to the south (Figure 1), suggesting that genetic variability decreases with increased isolation.

Table 8 presents an hierarchical analysis of F-statistics. By examining the relative values of the F-statistics it can be determined which hierarchical level contains the most genetic variability relative to the other levels. The results indicated that there is little regional differentiation within the total range. Most of

the genetic variability within the total sample was due to genetic differences between local populations; therefore, panmixis is geographically localized below the regional level. Intraregional Nm was estimated as 0.409; a value that implied that there was less than one migrant between populations in the same region every other generation. Within region X^2-tests for allele frequency heterogeneity corroborated this conclusion. Four of 13 polymorphic loci had significant levels of allele frequency heterogeneity among the two northern populations (PGM, p=0.01555; G6PDH, p=0.00001; PGD, p=0.03561; and GPI, p= 0.03357). Similarly, seven of nine polymorphic loci (AGP, p<0.001; AAT-1,2, p<0.001; MPI, p<0.001; PGM, p<0.001; G6PDH, p=0.00001; and MDH-2, p=0.00006) significantly differed in allele frequencies among the central samples. Two of four polymorphic loci (AGP, p<0.001; and GPI, p<0.001) also differed significantly in allele frequencies among the three southern samples. These results were expected given the low vagility of these butterflies (Gall, 1984a).

An UPGMA phenogram was derived from the genetic identities (Table 9) among all populations sampled (Figure 5). Samples within regions clustered most closely together, but genetic similarities among regional samples did not reflect their relative geographical positions. Samples from the northern and southern regions were more

genetically similar to one another than either was to the geographically intermediate central region.

"Private" alleles are defined as alleles that are segregating in one sample that are not present in any others. "Regional" alleles are those unique to a given region. The distribution of regional alleles revealed a pattern that would not be readily predictable from the present geographical distribution of the butterflies. Of the 20 loci examined, the number of regional alleles were distributed as follows: 1) North - seven, 2) Central - two, and 3) South - four (Figure 6). In addition, the north and central regions shared eight alleles not segregating in the south, central and south regions combined shared no alleles not found in the north, and north and south regions combined contained two alleles that were not segregating in the central region (Figure 6).

Table 7. Estimates of genetic variability at 20 allozyme loci in eight *B. improba* group populations (standard errors in parentheses).

Population	Mean No. of alleles per locus	Percentage of loci polymorphic*	Mean Heterozygosity	
			Observed	Expected
Haines Junction, YT	1.6 (.2)	50.0	.079 (.026)	.093 (.033)
Montana Mountain, YT	1.8 (.2)	55.0	.069 (.024)	.127 (.043)
Pink Mountain, BC	1.5 (.1)	40.0	.096 (.043)	.114 (.045)
Mount Hamell, AB	1.2 (.1)	20.0	.084 (.039)	.096 (.044)
Prospect Mountain, AB	1.1 (.1)	10.0	.041 (.033)	.041 (.033)
Bears Ears, WY	1.0 (.1)	5.0	.001 (.001)	.001 (.001)
Goat Flat, WY	1.1 (.1)	15.0	.014 (.001)	.013 (.010)
Red Cloud Peak, CO	1.1 (.1)	10.0	.022 (.018)	.031 (.025)

* A locus is considered polymorphic if the frequency of the most common allele does not exceed .99.

Table 8. Hierarchical F-statistics among *B. improba* group populations. See text for adescription of the hierarchy.

Comparison		F
X	Y	XY
POPULATION	REGION	.379
POPULATION	TOTAL	.486
REGION	TOTAL	.172

Table 9. Unbiased genetic distances (Nei, 1978) above diagonal and genetic identities (Nei, 1978) below diagonal among eight sampled *B. improba* group populations.

Population	A	B	C	D	E	F	G	H
A. Haines Junction, YT	*****	.013	.039	.062	.098	.021	.015	.059
B. Montana Mountain, YT	.987	*****	.045	.068	.123	.026	.023	.064
C. Pink Mountain, BC	.961	.956	*****	.065	.089	.096	.086	.143
D. Mount Hamell, AB	.940	.934	.937	*****	.038	.092	.089	.152
E. Prospect Mountain, AB	.906	.884	.915	.963	*****	.177	.166	.224
F. Bears Ears, WY	.979	.974	.908	.912	.838	*****	.000	.057
G. Goat Flat, WY	.985	.978	.917	.914	.847	1.000	*****	.055
H. Red Cloud Peak, CO	.943	.938	.867	.859	.800	.944	.947	*****

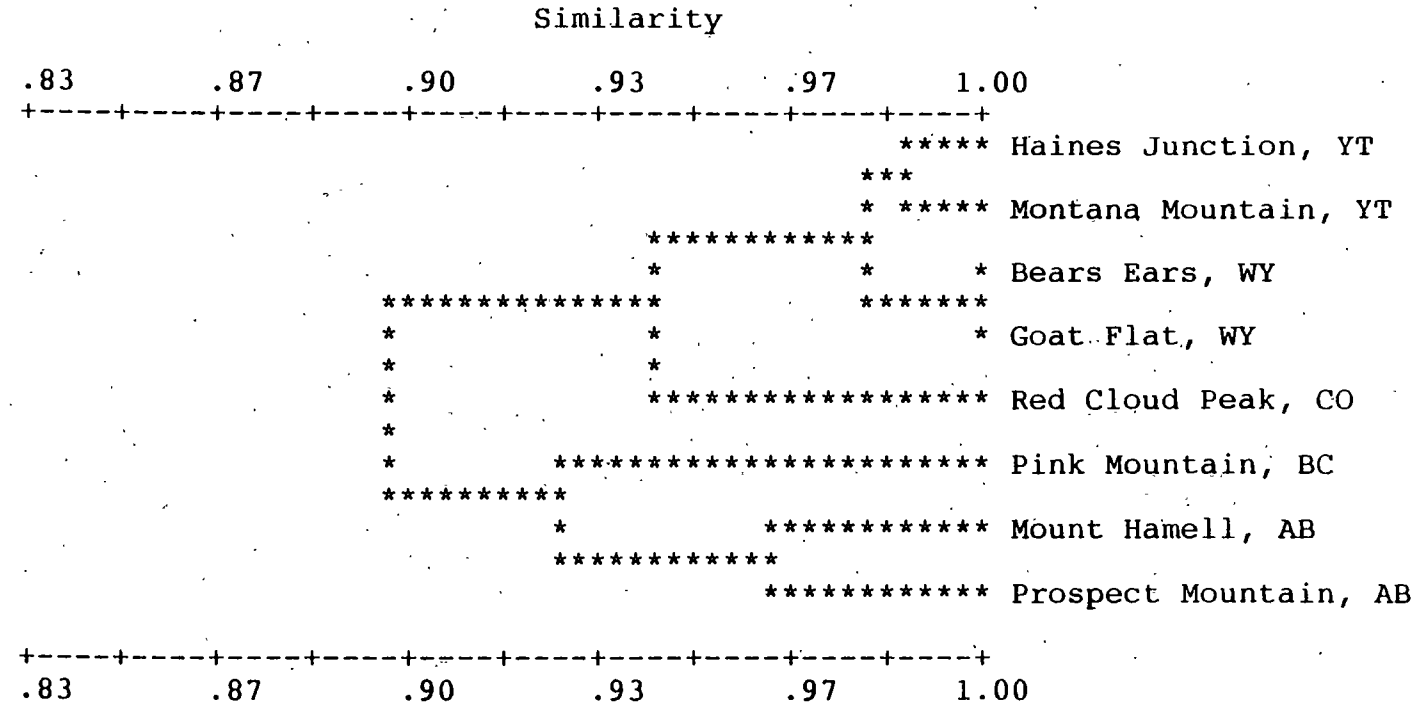

Figure 5. UPGMA phenogram based on unbiased genetic identities (Nei, 1978) for *B. improba* group populations. Cophenetic correlation coefficient =.737.

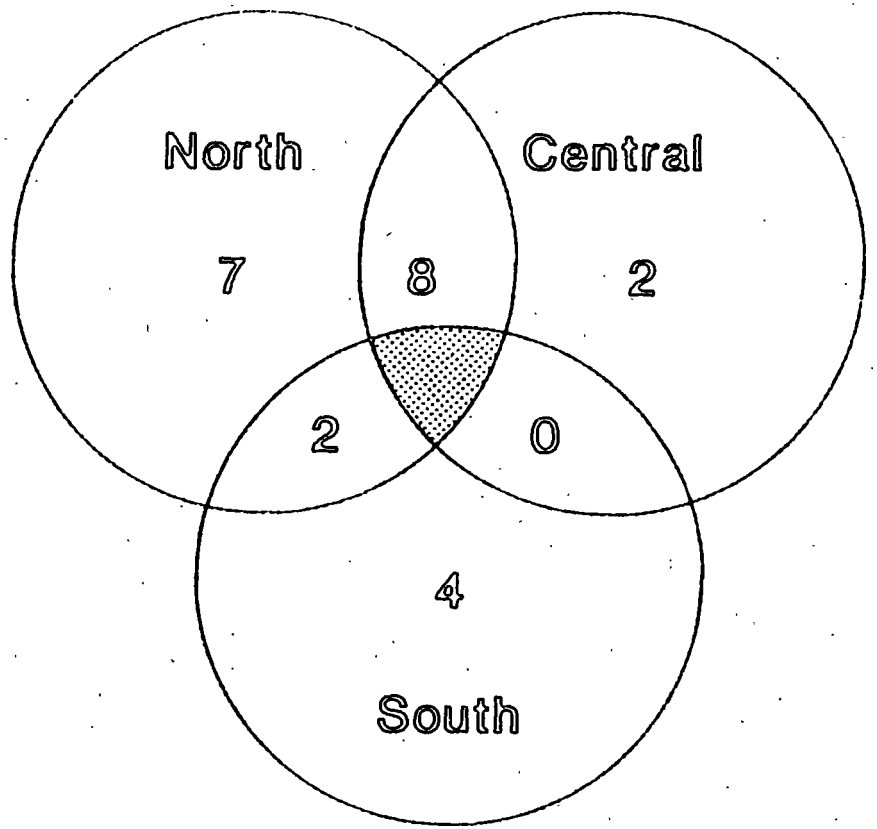

Figure 6. Circle diagram with numbers of unique regional alleles and alleles shared by pairs of regions in eight *B. improba* group colonies.

Boloria titania

Table 2 contains allele frequencies of the five *B. titania* populations sampled. Samples were pooled between years at sites where collections were made during more than one year. There were statistically significant deficiencies in numbers of heterozygotes in the Haines Junction, YT, (IDH-1, MPI, SOD, PGM, G6PDH, PGM, and MDH-2), Pink Mountain, BC, (IDH-1, AAT-2, and MDH-2), Uncompahgre Peak, CO, (PGM, G6PDH, and PGD), and Red Cloud Peak, CO, (PGD) samples. The Haines Junction, YT, sample had a significant pattern of heterozygote deficiency; of the 10 polymorphic loci in this sample, nine were heterozygote deficient ($p=0.0108$; Sign Test). Similarly, a nearly significant trend of heterozygote deficiency was detected in the Pink Mountain, BC, sample in which eight of 10 polymorphic loci were heterozygote deficient ($p=0.0547$; Sign Test).

Estimates of genetic variablility in *B. titania* populations are presented in Table 10. Unlike populations in the *B. improba* group, percentage of polymorphic loci was not significantly correlated with latitude ($r=-0.6669$, $p=0.1823$). No significant correlations were found among mean number of alleles per locus and latitude ($r=0.0513$, $p=0.9183$) or mean expected heterozygosity and latitude

(r=0.3696, p=0.5404). Nonhierarchical mean F-statistics across all 18 assayed loci for the five samples were: F_{is}=0.307, F_{it}=0.626, and F_{st}=0.461. The number of migrants per generation for these populations was estimated as Nm=0.292. This number was expected to be low given the large distances between sample sites. There were statistically significant differences in allele frequencies at 11 of the 15 polymorphic loci (IDH-1,2, p<0.001; AAT-2, p<0.001; MPI, p<0.001; SOD, p=0.00006; PGM, p<0.001; G6PDH, p<0.001; PGD, p<0.001; GPI, p<0.001; and MDH-1,2, p<0.001) among the five *B. titania* samples.

Table 10. Estimates of genetic variability in five *B. titania* populations. (Standard errors are in parentheses).

Population	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean Heterozygosity	
			Observed	Expected
Haines Junction, YT	2.4 (.4)	61.1	.108 (.031)	.213 (.062)
Pink Mountain, BC	2.1 (.3)	61.1	.167 (.062)	.223 (.059)
Prospect Mountain, AB	1.3 (.1)	27.8	.039 (.016)	.058 (.031)
Uncompahgre Peak, CO	2.4 (.3)	72.2	.102 (.034)	.136 (.043)
Red Cloud Peak, CO	2.3 (.3)	72.2	.121 (.045)	.155 (.053)

* A locus is considered polymorphic if the frequency of the most common allele does not exceed .99.

The genetic identities (Nei, 1978) in Table 11 were used to produce an UPGMA phenogram for the five *B. titania* populations sampled (Figure 7). Although the Colorado samples were closely clustered, samples from the central region (Prospect Mountain, AB, and Pink Mountain, BC) were widely separated. This was unlike the phenogram derived for the *B. improba* group in which samples within regions clustered most closely together.

The distribution of regional alleles over the 18 loci examined was: 1) North - six, 2) Central - three, and 3) South - 10 (Figure 8). Furthermore, when combined, the north and central regions shared five alleles not found in the south, central and south regional samples shared five alleles not found in the north, and the north and south regions shared four alleles not segregating in the central samples (Figure 8).

Table 11. Unbiased genetic distances (Nei, 1978) above diagonal and genetic identities (Nei, 1978) below diagonal for five *B. titania* populations.

Population	A	B	C	D	E
A. Haines Junction, YT	*****	.316	.215	.242	.252
B. Pink Mountain, BC	.729	*****	.322	.328	.333
C. Prospect Mountain, AB	.807	.725	*****	.076	.075
D. Red Cloud Peak, CO	.777	.717	.928	*****	.003
E. Uncompahgre Peak, CO	.785	.721	.927	.997	*****

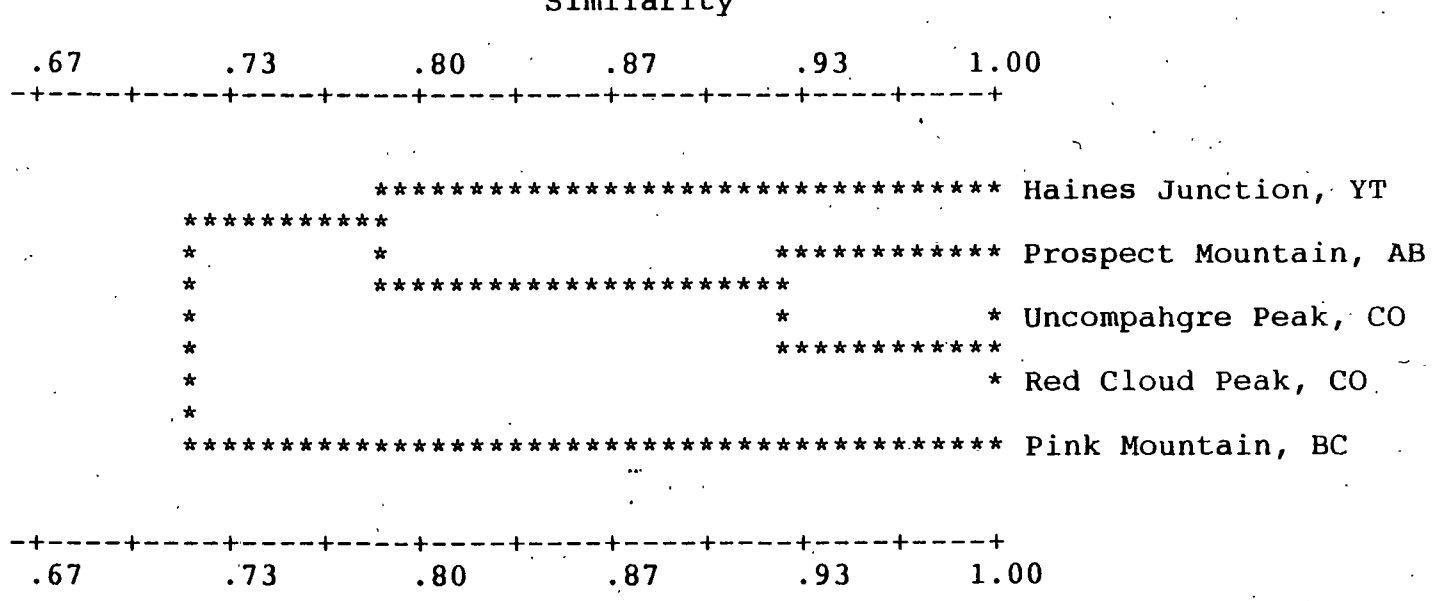

Figure 7. UPGMA phenogram based on unbiased genetic identities (Nei, 1978) for five *B. titania* populations. Cophenetic correlation coefficient=.997.

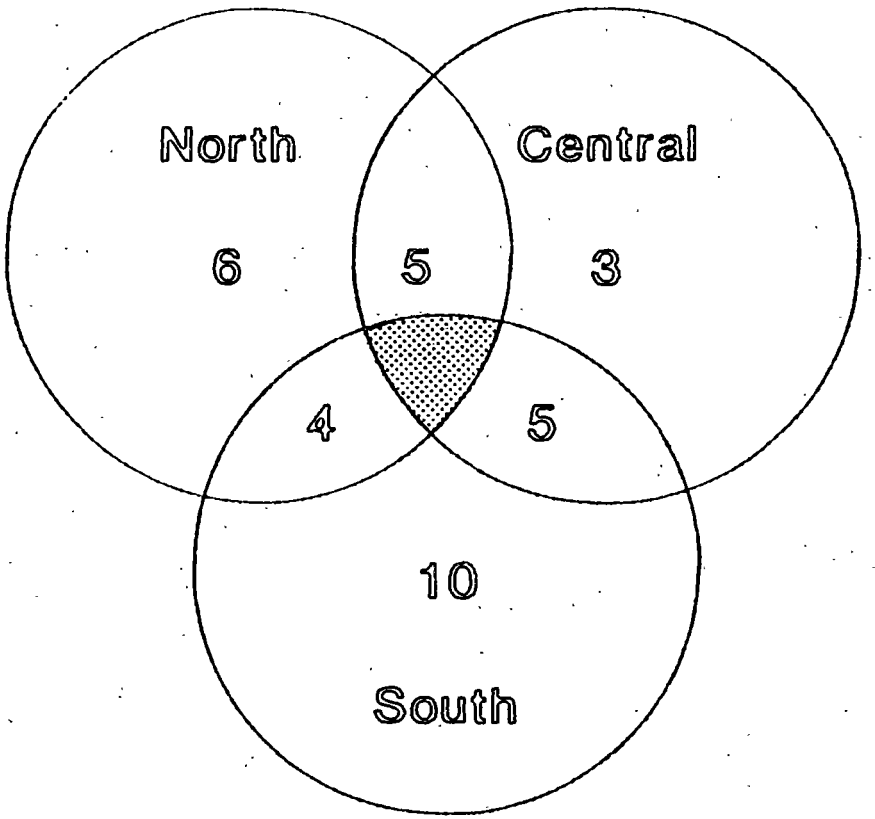

Figure 8. Circle diagram with numbers of unique regional alleles and alleles shared by pairs of regions in five *B. titania* colonies.

Habitat

The measured physical, cover and edge characteristics for each *B. improba* group site are given in Table 12. Six of 21 variables were significantly different among regions. These variables were: 1) standardized elevation (df=11, p=0.001), 2) aspect index (df=11, p=0.012), 3) non-food shrub cover (df=11, p=0.004), 4) large rock cover (df=11, p<0.01), 5) willow edge (df=11, p<0.01), and 6) meadow edge (df=11, p=0.036).

A plot of the first two principal components based on these six variables is presented in Figure 9. The five Colorado sites formed a tight cluster near the means of the two principal components with the other colony sites scattered around it (Figure 9). This implied that the five Colorado sites resembled one another closely with respect to the variables used. It also implied that *B. acrocnema* requires habitat near the mean of the habitat requirements of the *B. improba* group as a whole. Northern and central region sites formed loose clusters while the Bears Ears, WY, site more closely resembled the Colorado sites than the other Wyoming site (Goat Flat; Figure 9). Eigen-values and principal component loadings for the first two principal component axes indicated that standardized elevation, large rock cover, non-food shrub cover, and willow edge were the

most important variables determining site positions on the first principal component axis (Table 13). Similarly, aspect index, meadow edge, and willow edge were the most important variables on the second principal component axis. The first two principal components accounted for approximately 73% of the variance in the data.

Table 12. Habitat variables measured at 12 occupied *B. improba* group colony sites. Site designations are: 1--Haines Junction, YT, 2--Montana Mountain. YT, 3--Pink Mountain, BC, 4--Mount Hamell, AL, 5--Prospect Mountain, AL, 6--Bears ears, WY, 7--Goat Flat, WY, 8--Red Cloud Peak, CO, 9--Uncompahgre Peak, CO, 10--Uncompahgre Peak site 2, CO, 11--Alpine Gulch, CO, and 12--Baldy Chato, CO.

Site	1	2	3	4	5	6	7	8	9	10	11	12
Standardized Elevation (m)	3650	3765	3687	3737	3525	4162	3962	3854	3591	3890	3832	3945
Aspect	ENE	WNW	W	S	NW	E	WNW	NNE	NE	NNE	NE	NNE
Percent Slope	20	20	15	15	40	15	20	40	33	40	28	20
Site Length (m)	200	1000	300	250	200	300	1000	450	300	250	50	150
Larval Food Cover	12.45	4.17	4.45	30.96	5.12	16.91	8.20	15.90	28.90	16.20	14.00	4.60
Non-Food Shrub Cover	37.93	57.26	19.06	13.20	48.93	0.03	1.57	3.34	6.30	0.83	20.00	6.56
Forb Cover	10.80	8.72	36.82	8.33	16.11	22.17	24.91	19.20	31.50	24.60	38.60	42.60
Grass Cover	24.86	22.09	30.03	35.29	9.42	11.89	23.38	34.10	16.10	22.90	23.60	14.40
Moss Cover	0.03	0.72	1.89	0.00	3.94	0.39	0.00	1.30	3.50	1.10	6.70	1.90
Lichen Cover	0.08	0.01	0.54	0.87	0.00	0.03	0.00	0.30	0.00	0.09	0.00	0.11
Bare Soil Cover	0.03	0.03	0.21	3.34	0.42	0.28	1.31	11.90	0.81	18.80	5.50	4.10
Litter Cover	12.47	3.31	5.22	7.74	2.72	6.50	5.59	6.30	6.20	13.10	4.80	8.70
Small Rock Cover	0.01	0.00	1.18	0.00	12.93	5.60	9.52	4.50	2.50	2.80	4.00	4.60
Large Rock Cover	0.00	1.25	1.19	0.00	0.39	36.18	25.50	3.10	4.10	0.08	0.00	12.50
Willow Edge	70.00	55.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00	5.00	15.00	0.00
Meadow Edge	30.00	30.00	95.00	90.00	85.00	80.00	20.00	55.00	60.00	40.00	80.00	60.00
Talus Edge	0.00	0.00	5.00	0.00	0.00	0.00	70.00	10.00	0.00	0.00	5.00	40.00
Scree Edge	0.00	15.00	0.00	10.00	10.00	0.00	0.00	25.00	40.00	40.00	0.00	0.00
Rock Face Edge	0.00	0.00	0.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00
Snow Edge	0.00	0.00	0.00	0.00	5.00	10.00	10.00	0.00	0.00	15.00	0.00	0.00
Frequency of Nectar Sources	0.86	0.00	0.33	0.00	0.00	0.15	0.10	0.13	0.02	0.00	0.22	0.17

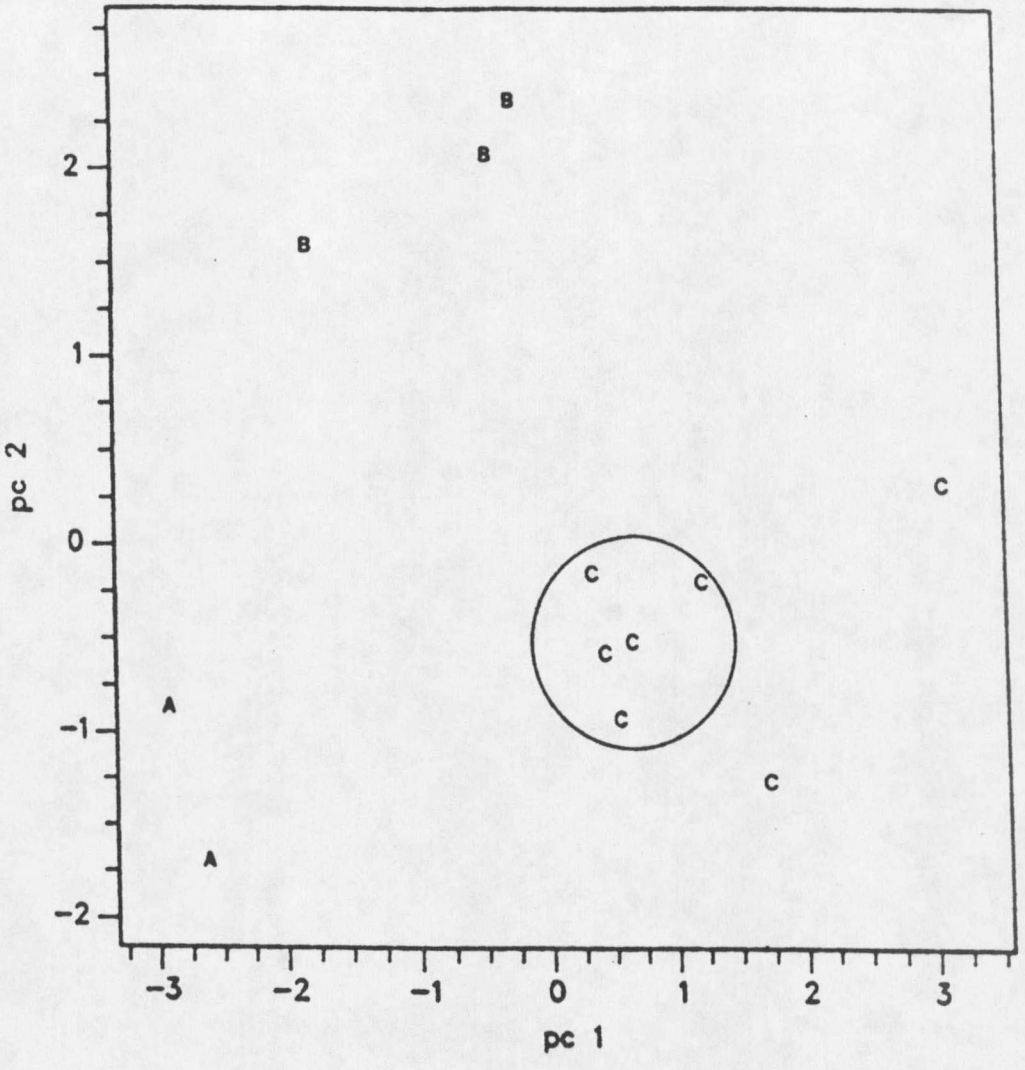

Figure 9. PCA plot of six habitat variables measured at 12 occupied *B. improba* group colony sites. Regional designations are: A--northern, B--central, and C--southern. Occupied *B. acrocnema* sites are circled.

Table 13. PCA results using six habitat variables from 12 occupied *B. improba* group colony sites.

Principal Component	1	2
Eigenvalue	2.759	1.597
Percent of variance accounted for by PC	45.986	26.662
Cumulative percent	45.986	72.608
Variable	Variable Loading	
Standardized elevation	.556	-.221
Aspect index	-.078	.518
Non-larval food shrub	-.537	.061
Large rock cover	.446	-.119
Willow edge	-.433	-.412
Meadow edge	.101	.704

Taxonomy

Measurement error did not appear to be a significant factor in the phenetic analysis of *B. improba* group wing characters. Standard errors of the eight continuous wing characters ranged from 0.008 mm to 0.066 mm with a mean of 0.028 mm. Discrete characters were scored consistently. Of the 24 repeated scorings made among the six individuals whose scores were replicated, only three mis-scorings occurred.

Figure 10 shows a plot of the PCA based on 12 wing characters as measured from *B. bellona* specimens and a sample of *B. improba* group individuals excluding *B.i. harryi*. *B. bellona* was separated from the *B. improba* group individuals along PC1, an axis on which forewing ground color and length were most heavily loaded (Table 14). *B. acrocnema* was separated from the rest of the *B. improba* group along PC2, an axis defined by diameter of the forewing postmedian spot in cell M_3 and the extent of purple coloration on the ventral surface of the hindwing (Table 14).

Figure 11 provides a plot of the PCA in which all sampled *B. improba* group colonies are represented. As in the previous PCA (Figure 10), *B. bellona* individuals were clustered separately from the *B. improba* group along PC1,

and forewing ground color and length were again the most heavily loaded characters on this axis (Table 15). There was, however, less separation of *B. acrocnema* from the rest of the *B. improba* group along PC2 due to the inclusion of *B.i. harryi* (Figure 11). The loadings on PC2 in this analysis indicated that the extent of white coloration on the dorsal surface of the hindwing and the extent of purple coloration on the ventral hindwing surface accounted for most of the structure in the data along this axis (Table 15). Therefore, *B.i. harryi* was apparently intermediate between other *B. improba* subspecies and *B. acrocnema* with respect to the measured characters.

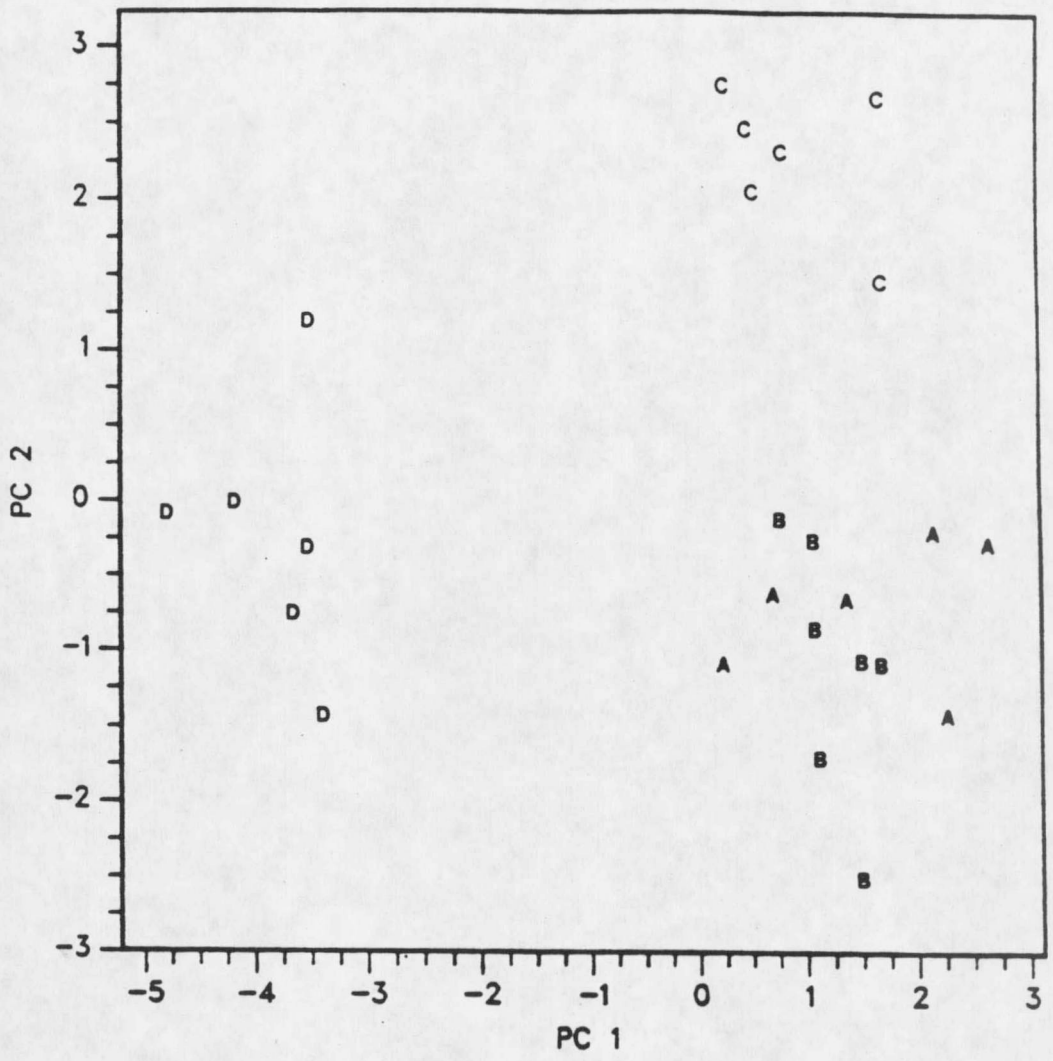

Figure 10. PCA plot of 12 wing characters measured from 19 *B. improba* group individuals excluding *B.i. harryi*, and six *B. bellona* specimens. Letter designations are: A--northern *B. improba*, B--central *B. improba*, C--*B. acrocnema*, and D--*B. bellona*.

Table 14. Results of PCA on wing characters measured from *B. bellona* and *B. improba* group specimens excluding *B.i. harryi*.

Principal Component	1	2
Eigen value	5.010	2.127
Percent of variance accounted for by PC	41.751	17.726
Cumulative percent	41.751	59.477
Variable	Variable Loading	
FW length	-.427	-.097
FW ground color	.389	-.073
Extent of white on FW	.165	.481
Width of FW postmedian spot in cell M_3	.058	.510
Width of postmedian spot in cell R_5	-.139	.184
Distance from FW postmedian spot in cell R_4 to costal margin	-.324	-.142
Distance from FW postmedian spot in cell M_2 to outer margin	-.183	.318
Width of FW postmedian band in cell M_3	-.391	-.217
Extent of HW melanization	.354	-.226
Length of HW postmedian-median discal spot	-.215	.155
Distance from HW postmedian-median discal spot to outer margin	-.355	-.059
Extent of purple on HW	.167	-.461

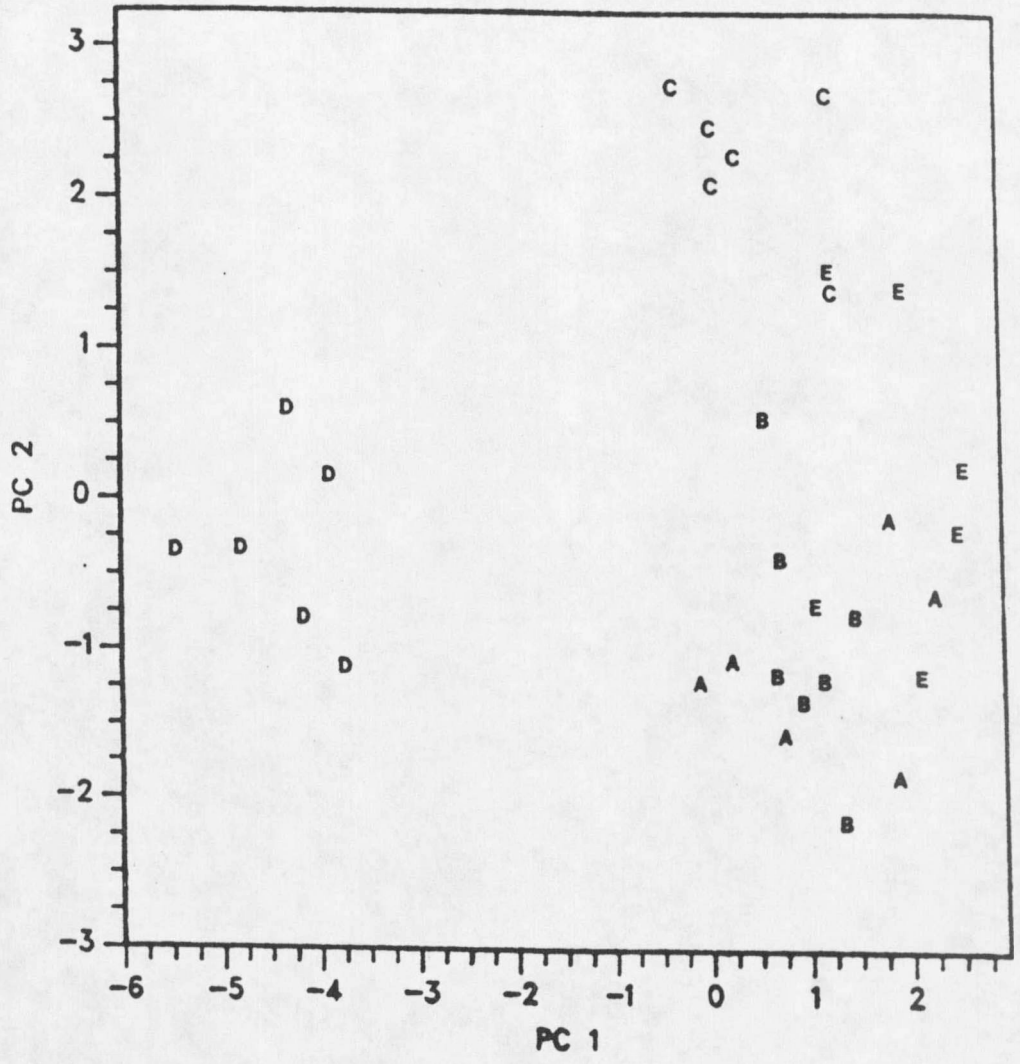

Figure 11. PCA plot of 12 wing characters measured from 25 *B. improba* group individuals and six *B. bellona* specimens. Letter designations are: A--northern *B. improba*, B--central *B. improba*, C--*B. acrocnema*, D--*B. bellona*, and E--*B.i. harryi*.

Table 15. Results of PCA on wing characters measured from *B. bellona* and *B. improba* group specimens.

Principal Component	1	2
Eigen value	5.034	1.918
Percent of variance accounted for by PC	41.951	15.985
Cumulative percent	41.951	57.936
Variable	Variable Loading	
FW length	-.426	-.111
FW ground color	.369	-.139
Extent of white on FW	.183	.525
Width of FW postmedian spot in cell M_3	-.031	.341
Width of postmedian spot in cell R_5	-.139	.016
Distance from FW postmedian spot in cell R_4 to costal margin	-.335	-.148
Distance from FW postmedian spot in cell M_2 to outer margin	-.231	.242
Width of FW postmedian band in cell M_3	-.387	-.242
Extent of HW melanization	.319	-.265
Length of HW postmedian-median discal spot	-.178	.259
Distance from HW postmedian-median discal spot to outer margin	-.363	-.156
Extent of purple on HW	.173	-.525

DISCUSSION

Population Trends

B. acrocnema populations have apparently been declining at both colony sites in the San Juan Mountains since the early 1980's. The decline of *B. acrocnema* is traceable using CMR estimates from Gall (1984a), this study, and anecdotal evidence from U.S. Forest Service files. The biennial life cycle of *B. acrocnema* divides even-year and odd-year broods into separate populations, although there is, undoubtedly, some "leakage" between broods (e.g. some larvae may take three rather than two years to develop).

Gall (1984a) estimates that between 650 and 750 *B. acrocnema* flew at Uncompahgre Peak, CO, in 1980. Zieroth (Forest Service files) estimated that about 500 butterflies were present at the type locality in 1982. There are no estimates available for the 1984 and 1986 Uncompahgre Peak populations. Approximately 200 *B. acrocnema* flew at Uncompahgre Peak in 1988, and no *B. acrocnema* were seen in flight at Uncompahgre Peak in 1990 (Seidl, 1990).

The odd-year brood at the type locality has fared even worse. Dr. James Scott (letter to Forest Service; 30 July 1980) estimated the 1979 population size at Uncompahgre

Peak to be about 200. Zieroth (1981) reported that this colony was "greatly reduced" during the 1981 flight season. William Shuster, a Forest Service wildlife biologist, saw "few" butterflies at Uncompahgre Peak in 1983 and reported seeing only three butterflies at the site in 1985 (Forest Service memo; 3 October 1985). No odd-year brood butterflies were seen in 1987.

Both *B. acrocnema* broods at Red Cloud Peak, CO, have evidently been more robust than the Uncompahgre Peak populations, but there is a general decline in population size estimates at this site as well. The Red Cloud Peak colony was discovered in 1982 (Gall, 1984a). Gall estimated its size at between 1,000 and 1,500 individuals that year (pers. com. to P. Brussard). Approximately 200 *B. acrocnema* flew at this site in 1988. There are no population size estimates for the intervening even-years at Red Cloud Peak. Information for the odd-year brood at Red Cloud Peak is even more sketchy. Butterflies were apparently present at the site in 1983 and were "doing well" in 1985 (BLM memo; J. Capodice, 17 April 1986). There were 250-300 butterflies at Red Cloud Peak during the 1987 flight season.

Although much of the evidence is anecdotal and many of the population size estimates from CMR studies may not be reliable on theoretical grounds, these estimates provide convincing evidence that there has been a general decline

in *B. acrocnema* numbers at the two known colony sites. The butterfly apparently no longer exists at its type locality, so the Red Cloud Peak colony is the only remaining relatively large population of this species. Even it is declining rapidly. However, *B. acrocnema* has been found in small numbers at three other sites in the San Juan Mountains, two of which are near the known colonies. It is possible that other large colonies exist in SW Colorado, but a search of over 50 potential sites in and near the San Juans over the course of two years failed to discover any. Federal personnel and private collectors have spent much time since the discovery of *B. acrocnema* looking for other colonies and also have not found any.

The tenets of population viability analysis (PVA) provide insight into the decline of *B. acrocnema*. In the absence of deterministic causes (e.g. systematic habitat destruction), the persistence of a given population is largely determined by stochastic factors (Brussard, 1986). Five factors are considered important to population viability: 1) demographic stochasticity, 2) environmental stochasticity, 3) infrequent and unpredictable catastrophes, 4) genetic stochasticity, and 5) metapopulation structure (Brussard, 1986; Gilpin and Soule', 1986; and Shaffer, 1987).

Demographic stochasticity is the random variation in birth and death events in a population. This type of

uncertainty occurs at the level of the individual. Demographic stochasticity becomes a threat to the persistence of a species only when there are very few individuals (10's to 100's) remaining (Brussard, 1986; and Shaffer, 1987). For an extreme example, consider a population with only two breeding females. It is quite possible that, due to the uncertainties involved in fertilization, both could have all male offspring in one breeding season. This outcome would seriously affect the ability of this hypothetical population to increase even under the best of environmental conditions.

Although the Red Cloud Peak, CO, *B. acrocnema* population is small, its numbers are probably not yet small enough for demographic uncertainty to play a major role in its future survival. Given that demographic stochasticity is often the immediate precursor of population extirpation (Gilpin and Soule', 1987), however, the continued decline of this population over the short-term could make this type of uncertainty very important to the persistence of the Red Cloud population. The Uncompahgre Peak even-year brood may be a good example of how rapidly these forces can cause the loss of populations.

Environmental stochasticity is random environmental variation which affects population-wide birth and death probabilities and is an important cause of population decline (Brussard, 1986; Gilpin and Soule', 1987; and

Shaffer, 1987). This type of uncertainty has probably been the key factor in the decline of *B. acrocnema* populations in recent years, as it may have been for several other butterfly species (Ehrlich *et al.*, 1980). Specifically, mean annual temperatures were, on average, 0.67^{o} C above the long-term norm and mean annual precipitation was 2.8 mm below normal in SW Colorado in the 1980's (National Oceanic and Atmospheric Administration climatological data). Therefore, it is quite possible that environmental uncertainty in the form of several warm and dry years could have contributed to the decline of these populations.

Ehrlich *et al.* (1980) state that the ecological events that result in population size changes in butterflies are those that are associated with changes in quality and quantity of larval food sources. Climatically driven changes in *Salix nivalis* phenology could have a profound effect on the ability of *B. acrocnema* to complete its life cycle in the relatively short seasons available at high elevations. Larval growth rates may also be directly affected by such environmental variables as temperature and moisture conditions (Scott, 1986). Zieroth (1981) attributed the decline in the Uncompahgre Peak colony in 1981 to an unusually low winter snowfall and a warm spring. This event could have started the odd-year brood at Uncompahgre Peak on its decline. A parallel effect at Red

Cloud Peak would support this hypothesis, but the data are not available.

The biennial life cycle of *B. acrocnema* may buffer its populations against environmental uncertainty to some extent. There is apparently some leakage between odd-year and even-year broods since they have not significantly diverged genetically. Thus, a declining brood may be augmented both genetically and demographically by leakage between broods. It is also possible that an extirpated brood could be re-established by "migrants" from the off-year brood. This potential form of "rescue" (Brown and Kodric-Brown, 1977) was apparently not sufficient to prevent the demise of *B. acrocnema* at Uncompahgre Peak.

Catastrophic events can be viewed as extreme forms of environmental uncertainty. Catastrophes can be human caused, such as collecting or destruction of habitat, or they can be naturally caused (e.g., by fires and floods). Shaffer (1987) considers catastrophes an important cause of extinction because large increases in population size may offer little additional protection against catastrophic extinction.

There are several potential catastrophes that could cause extirpation of *B. acrocnema* from its colony sites. Gall (1984a) considers sheep grazing on these sites the most serious threat to these populations. Massive habitat loss due to trampling by a large number of sheep could

eliminate the butterfly from the grazed area (Gall, 1984a). Although there are sheep allotments on both *B. acrocnema* sites, no sheep have been grazed on them since the discovery of the colonies. The Bureau of Land Management diverted a large sheep drive from the Red Cloud Peak site in 1988, thus averting a potentially catastrophic event which could have resulted in the loss of the even-year brood at Red Cloud Peak.

Grazing by pack horses, especially at Uncompahgre Peak, may pose a similar threat. Eight horses were observed on the site on 27 August 1987. The horses had been tethered on the downslope end of the site while the riders hiked to the peak. Although this particular party does not visit Uncompahgre Peak regularly, such activity may occur frequently since the site is the last relatively level area along the trail before the climb to the peak. The potential damage to the site from several horses per week could be great; however, no other horses were observed at this site in 1987 and 1988.

Collecting poses another threat to the persistence of *B. acrocnema* populations (Gall, 1984a). Collectors were seen at both *B. acrocnema* sites in 1987 and 1988 despite bans on collecting by the federal agencies with jurisdiction over the sites. There is some question as to the effect of collecting on butterfly populations. Pyle *et al.* (1981) could find no documented cases of extinctions or

even local extirpations of insects due to collecting alone. The deleterious effects of collecting may be ameliorated by the phenology of *B. acrocnema* broods. Males emerge first in the flight season and patrol for eclosed females (Scott, 1986); thus, collecting males at any time in the flight season is unlikely to affect recruitment to a great extent. Collecting females, however, especially in large numbers from small populations, has the potential to affect demography. Since older, and probably mated, females tend to disperse (Gall, 1984a), their removal could also affect the establishment of new colonies.

Recreational hikers were mentioned by Gall (1984a) as a potential threat to *B. acrocnema* colonies. The trail through the Uncompahgre Peak site and the one near the Red Cloud Peak site are both heavily used by hikers; people were seen on these trails nearly every time work was being done at the sites. There is little reason for hikers to leave the trail at either locality, however, so the likelihood of adverse impact on the butterflies by this source is probably small.

Genetic stochasticity are the effects on short- and long-term fitness resulting from the random loss of alleles and inbreeding. Inbreeding is considered the greatest threat to short-term persistence, while the genetic variability needed for long-term adaptability can be lost through genetic drift (O'Brien *et al.*, 1985; and Shaffer,

1987). Several modeling studies using data from large-bodied tropical species have revealed that genetically effective population sizes of about 50 are needed for short-term persistence and about 500 genetically effective individuals per generation are needed for long-term survival (Shaffer, 1987). The applicability of these results to *B. acrocnema* is not known. Furthermore, genetically effective populations sizes are difficult to estimate from simple population size estimates because many variables may affect them (e.g., sex ratio, variance in family size, and variation in population size over time). It would appear, however, that the short-term persistence of *B. acrocnema* may be threatened by inbreeding given the high estimated mean within-colony inbreeding coefficient and the deficiencies of heterozygotes detected at the assayed loci. Furthermore, its probability of long-term persistence is probably threatened by its general lack of genetic variability.

Metapopulation structure, the distribution of local populations in time and space, may also have an impact on the survival of *B. acrocnema*. Wilson (1975) defined the concept of the metapopulation as a series of local populations some of which are going extinct while colonizers are re-establishing others. Thus, an equilibrium is reached in which the number of populations is constant but the occupancy state of any given site can

change (Wilson, 1975). Gall (1984a) assumes a metapopulation structure for *B. acrocnema*. Given the philopatric nature of male and early season female butterflies, dispersing late season females are probably the most important element of colonizers in the population (Gall, 1984a). Ehrlich *et al.* (1980) suggest that *Euphydryas* ssp. may also exist as metapopulations. Extinction of an entire species with a metapopulation structure would probably only occur after a large proportion of local populations succumb to the uncertainties of demography or the environment. When the rate of recolonization is exceeded by the rate of local population loss, the species is in danger of extinction.

The apparent move toward extinction of *B. acrocnema* probably cannot be explained by one factor alone. It is likely that climatic changes have played a large role by reducing population-wide reproduction and survival. The exact mechanisms by which drier and warmer conditions have impacted these populations are not known, although it is reasonable to expect that the delicate balance between larval food plant phenology and larval development has been disrupted. For example, earlier than normal spring-like conditions could provide cues to larvae to break diapause before *S. nivalis* plants are in suitable post-winter condition for larval consumption. Under this scenario, larvae that are active too early would starve.

Assuming that climate change could suppress population growth further, if not eliminate some colonies outright, the remaining populations could be eliminated by a variety of demographic and environmental factors such as those discussed above. As local populations are extirpated, the pool of potential migrants could be diminished to the point at which new colonies are no longer being founded and previously occupied sites are not being recolonized. Once this occurs, the loss of each colony brings the species one step closer to extinction.

Detailed knowledge of population trends among other members of the *B. improba* group is nonexistent. This is unfortunate since it would provide a means to assess the hypothesized causes for the decline in *B. acrocnema* numbers. Some daily population estimates were made for *B. improba* in the Yukon Territory and Wyoming, but not much can be concluded from them. The vagaries of weather conditions in the field and the priority placed on collecting for allozyme surveys coupled with the logistical difficulties of long distances between sample sites and short flight seasons often made CMR studies impractical. When attempted, CMR results were often unsatisfactory due to extremely low recapture rates, especially in northern populations.

Genetics

Allozyme Variability and Conservation Biology

The electrophoretic data compiled for this study provide a means of inferring the population structure and historical biogeography of the *B. improba* group. This approach requires that the assumptions of the neutral allele theory be met i.e., that mutation, drift, and migration are the principal processes by which genetic variability arises and is maintained in populations (Nei, 1983 and 1987). In contrast, when estimates of heterozygosity and other measures of genetic variability derived from allozyme data are used to make inferences about the survival of *B. improba* group colonies, a selectionist argument is invoked. Under this theory, natural selection is assumed to be the dominant process by which genetic variability is maintained in populations through overdominance, environmental heterogeneity, and other phenomena (Hartl, 1980). Thus, a paradox arises in which the same data set is assumed to have apparently contradictory properties.

The presumed nature of electrophoretic samples provides a resolution of this paradox. Any eletrophoretic sample examines only a small but representative fraction of the genome (Beardmore, 1983; Soule', 1979; Utter *et al.*,

1987; and Zink *et al.*, 1985). Although the possibility of some selection occurring at a few protein loci usually cannot be discounted, selective neutrality is probably the rule at most of these loci. This assumption is usually not violated to the point where population structure cannot be inferred from allozyme data in most studies (Nei, 1983; and Utter *et al.*, 1987). In addition, drift becomes more important than selection as effective population size decreases (Beardmore, 1983; Hartl, 1980; and Crow, 1986). Therefore, allozyme data from small populations, the ones usually of most interest in conservation biology, are even less likely to violate the assumption of neutrality than data from larger populations.

On the other hand, several studies have demonstrated a positive correlation between protein heterozygosity and components of fitness such as survival, fecundity, and developmental stability (Allendorf and Leary, 1986; Leary *et al.*, 1983; O'Brien *et al.*, 1985; and Palmer and Strobeck, 1986). It should be noted, however, that these results are not always consistent (Allendorf and Leary, 1986; and Zink *et al.*, 1985). The positive correlation between protein heterozygosity and fitness has two possible causes: 1) isozyme heterozygosity estimates are representative of genetic variability throughout the genome and, therefore, some of the protein loci are themselves involved in the expression of fitness related traits, and

2) electrophoretic variants are linked to genes contributing genetic variance to fitness related traits (Allendorf and Leary, 1986; and Zink *et al.*, 1985). In any case, protein heterozygosity estimates can be useful in inferring probabilities of population persistence because of their empirically demonstrated link with fitness. Thus, as Beardmore (1983) states, "the extent to which genetic variation in populations is maintained by deterministic or stochastic processes . . . is not a matter of critical importance" in conservation biology.

Within Colony Structure

Several conclusions about population structure within *B. improba* group colonies and gene flow between colonies can be reached using allozyme data. The deviation from Hardy-Weinberg expectations in the Haines Junction, YT, and Red Cloud Peak, CO, *B. improba* and *B. acrocnema* samples can be attributed to random sampling error; at a significance level of 0.05 one in 20 comparisons would be expected not to conform to Hardy-Weinberg expectations by chance alone. However, larger proportions of loci with heterozygote deficiencies in a sample require further explanation. There are five possible reasons why electrophoretic samples are deficient in heterozygotes: 1) scoring of heterozygotes as homozygotes, 2) null alleles segregating in the population, 3) selection against heterozygotes, 4)

inadvertent sampling of two or more semi-isolated demes which are statistically treated as a single panmictic unit (Wahlund effect), and 5) inbreeding.

Since the buffer systems and staining procedures used provided high enough resolution for unambiguous scoring it is unlikely that systematic scoring errors occurred over a large number of loci. The potential presence of a null allele segregating at a particular locus is difficult to disprove. In order to be detected, the null allele must be present in high enough frequencies to occur as a homozygote in several samples. This was not the case with respect to any of the loci that were found to be heterozygote deficient. Null alleles at low frequencies could be occurring in heterozygotes at these loci, but it is unlikely that they are frequent enough to cause the observed heterozygote deficiencies.

For example, consider a hypothetical sample of 50 diploid individuals in which a null allele with a frequency of 0.1 is segregating with two other alleles having frequencies of 0.5 and 0.4. If the null allele is undetectable and the other two alleles are assumed to have equal frequencies, 25 heterozygous individuals would be expected under Hardy-Weinberg assumptions while 27 would be expected if the null allele were detectable. The difference between numbers of expected heterozygotes is insignificant under these two scenarios ($p>0.5$; X^2-Test).

Negative heterosis cannot be disproven based on the data gathered, but selective neutrality or near neutrality is usually assumed for electrophoretic variants (Nei, 1983 and 1987).

The Wahlund effect is the most likely reason for the observed heterozygote deficiencies at these loci (Hartl, 1980). It can occur in behaviorally structured populations or when nonvagile organisms are sampled over a large area. In the case of the *B. improba* group, an area of over 1 km^2 was sampled at Montana Mountain, YT, and butterflies were collected from an area of several hundred square meters on Pink Mountain, BC. Given the very small movements of *B. acrocnema* in Colorado (Gall, 1984a) it is reasonable to assume that *B. improba* has similarly low vagility. Comparably small movements have also been observed in populations of other butterfly species (e.g. *Colias spp.*; Watt *et al.*, 1977 and *Euphydryas editha*; Gilbert and Singer, 1973). Likewise, *B. titania* were collected over even larger areas than *B. improba* because they occur in somewhat lower densities in the areas sampled. Thus, several semi-isolated demes may have been sampled at these localities. Different allele frequencies in those demes could result in the observed heterozygote deficiencies relative to panmixia throughout the sampled areas (Hartl, 1980).

The relatively high estimate of mean within-sample inbreeding (F=0.159) indicates that inbreeding, or inbreeding-like effects, may also account for some of the observed deficiencies in heterozygotes in *B. improba* group samples. Actual matings between related adults would require that two conditions be met: 1) related adults would have to eclose within a few days of each other after two years of development, and 2) they would have to eclose in close proximity to one another. Given the very short flight season of these butterflies and their apparently sedentary nature (Gall, 1984a), these conditions could potentially be met in *B. improba* group populations. A very detailed behavioral study, combined with allozyme data, would be required to determine the relative strengths of inbreeding and Wahlund effect in maintaining the very small-scale population structure that is indicated in *B. improba* group colonies. The best conclusion that can be reached with the present data is that the low vagility of these short-lived butterflies may result in fairly frequent matings among close relatives.

Between Colony Gene Flow

Gene flow is apparently restricted within very localized demes in *B. improba* group colonies as evidenced by the detection of a probable Wahlund effect in several of the populations sampled. Gene flow between populations

therefore is expected to be restricted as well. F-statistics partition the total genetic variance in a population into the variance within subpopulations and the variance between subpopulations (Hartl, 1980). F-statistics can also be used in hierarchical situations in which populations are grouped within regions and all regions together form the total sample. Hierarchical F-statistics for the *B. improba* group (Table 8) indicate that much of the total genetic variance is held within individual populations. Regions are not differentiated as much as are the individual populations within them. Porter and Geiger (1988) state that, under the assumption of selective neutrality, F_{st} values that exceed 0.333 imply that little present-day gene flow is occurring among the sampled populations. The population-region F-statistic (F_{xy}=0.379; Table 8) and the low estimate of Nm (0.409) indicate that there is little gene flow between *B. improba* group populations that are in the same region. Genetic drift may be an important factor in the differentiation of these populations given the apparently low level of gene flow between them (Slatkin, 1987). High F_{st} values along with very low estimates of Nm imply that gene flow between *B titania* colonies is at least as limited as it is between *B. improba* group colonies. Statistically significant heterogeneity in allele frequencies among *B. improba* group populations in the same regions and among *B. titania*

populations also provide evidence that interpopulation gene flow is restricted.

Relatively large F_{st} values and significant heterogeneity in allele frequencies between populations have been documented in a number of other taxa as well (Eanes and Koehn, 1978). Significant levels of genetic heterogeneity have been found among Scandinavian moose (*Alces alces*) populations that are as little as 50 km apart (Chesser *et al.*, 1982). Significant levels of population differentiation have also been noted among pocket gopher (*Thomomys talpoides*; Patton and Feder, 1981) and black-tailed prairie dog (*Cynomys ludovicianus*; Chesser, 1983) populations. Most of the cases of genetic differentiation among mammalian populations have been attributed to genetic drift occurring in socially isolated breeding units which usually have small effective population sizes (Patton and Feder, 1981; and Chesser, 1983). Other studies (e.g., McCracken and Brussard, 1980; Selander and Kaufman, 1975; and Selander and Hudson, 1976) have demonstrated that high levels of genetic heterogeneity can occur between populations of relatively non-vagile animals such as snails. Both stochastic processes and selection may determine the genetic composition of each isolated population (McCracken and Brussard, 1980).

Gall (1984a) determined that *B. acrocnema* are extremely sedentary. From allozyme data, one may infer

that there is little gene flow among populations in the *B. improba* group as a whole. The non-vagile nature of these species has apparently resulted in restricted gene flow within and between colonies. Low levels of gene flow between *B. improba* group populations may explain the decreases in estimated genetic variability that occur with decreasing latitude. Central and southern region populations in the *B. improba* group are increasingly more geographically isolated to the south (Figure 1). Indeed, populations in the southern region (*B.i. harryi* and *B. acrocnema*) are completely disjunct from the rest of the group (Figure 1). This geographical isolation is reflected in the progressive decrease in genetic variability of *B. improba* group populations in the southern part of its distribution.

Relative Variability

Estimates of genetic variability in northern *B. improba* group populations (Table 7) fall within the range reported for non-*Drosophila* spp. insects in general and butterfly species in particular. Hartl (1980) reports an average heterozygosity level of 0.151 and a 53.1% polymorphism level among four insect species, excluding *Drosophila* spp., at 18 allozyme loci. Crow (1986) reports that the average heterozygosity of non-*Drosophila* spp. insect populations is 0.089 as estimated in a number of allozyme surveys. *Drosophila* spp. were excluded from these studies because of

their atypically high levels of genetic variability relative to other insects. Porter and Geiger (1988) estimate that the mean heterozygosity of *Coenonympha tullia* populations in the western U.S. ranges from 0.123 to 0.185 and that the percentage of polymorphic loci in these populations falls between 32.9 and 58.8. Brittnacher *et al.* (1978) estimate that the average heterozygosities of a number of species within the genus *Speyeria* range from 0.067 to 0.149. Kitching (1988) reports mean heterozygosity levels of 0.10 to 0.25 in a number of Daniad butterfly populations. Finally, Brussard and Vawter (1975) estimate mean heterozygosity levels of 0.22 to 0.72 and polymorphism levels of 43% in *Euphydryas phaeton* populations.

Central and southern *B. improba* group populations have generally lower levels of genetic variability than those estimated for other butterfly species (Table 7). Significant correlations between estimates of genetic variability and latitude indicate that genetic variability decreases from north to south in *B. improba* group populations. In contrast, all but the Prospect Mountain, AB, *B. titania* population fall within the range of genetic variability reported for butterfly species in other studies (e.g., Porter and Geiger, 1988; Kitching, 1988; and Brittnacher *et al.*, 1978). Additionally, estimates of

genetic variability and latitude are not significantly correlated in *B. titania* populations.

Population Heterozygosity

Several studies (e.g., Wade *et al.*, 1983; Kilpatrick and Crowell, 1985; and Ayala *et al.*, 1971) have documented reductions in mean heterozygosity and allozyme polymorphism in geographically isolated populations of many taxa. Random processes can result in the loss of neutral alleles from populations that receive few dispersers to replace them (Crow, 1986; and Hartl, 1980). Inbreeding in small, isolated populations can further reduce heterozygosity. Isolated populations may also experience unique selection pressures. This can reduce genetic variability in these populations as well (Hartl, 1980; Wade *et al.* 1983; and Ayala *et al.*, 1971). Since selective neutrality is usually assumed for electrophoretic variants (Nei, 1983 and 1987), it is reasonable to expect that stochastic processes are the most likely causes of the loss of variability in the southern *B. improba* group populations.

There are several important implications of restricted gene flow and low levels of genetic variability for the long-term persistence of *B.i. harryi* and *B. acrocnema*. The advantages of genetic variability have been documented in a number of studies (e.g. Allendorf and Leary, 1986; O'Brien *et al.*, 1985; and Watt *et al.*, 1983). These studies have

concluded that heterozygous individuals may have higher fitnesses than homozygous individuals and that genetically variable populations should be better able to adapt to changing environmental conditions, and thus persist longer than less variable populations (Allendorf and Leary, 1986; and O'Brien *et al.*, 1985).

Allendorf and Leary (1986) surveyed 21 studies in the current literature concerning the effects of heterozygosity on survival. In 15 of the studies surveyed it was determined that heterozygous individuals have an advantage over homozygous conspecifics for this component of fitness (Allendorf and Leary, 1986). Positive relationships between heterozygosity and disease resistance, high growth and development rates, and developmental stability have also been demonstrated (Allendorf and Leary, 1986). Watt (1983) presents an example of heterzygote superiority at the PGI locus in *Colias* spp. butterflies. Heterozygotes had the highest *in vitro* substrate binding affinities, and thus the greatest efficiencies, under a variety of pH and temperature conditions (Watt, 1983). When tested in the field, PGI heterozygotes were able to fly at lower temperatures than homozygotes; thus, heterozygous individuals were able to initiate flight earlier in the day and to fly for longer durations than their homozygous conspecifics (Watt, *et al.*, 1983). These heterotic effects

could have implications for the survival, and therefore fitness, of individual *Colias* spp. butterflies.

O'Brien *et al.* (1985) convincingly demonstrated that extremely low levels of heterozygosity and polymorphism in southern African cheetah (*Acinomyx jubatus*) populations are associated with susceptibility to disease, male infertility, and high juvenile mortality. The lack of significant amounts of genetic variability in the cheetah implies that the species may be unable to adapt to environmental changes, therefore it is in danger of extinction (O'Brien *et al.*, 1985). A similar investigation of cheetahs in east Africa and elsewhere would help confirm these conclusions (O'Brien *et al.*, 1985).

Although southern *B. improba* group populations are considerably more variable than cheetah populations, they are significantly less so than northern *B. improba* group populations. Given the low vagility of these butterflies and their disjunct distribution, it is unlikely that gene flow will significantly augment these isolated gene pools. This may be particularly important in *B. acrocnema* since the only two known colonies of this butterfly have been declining in numbers over the last decade. If *B. acrocnema* can survive the current bottleneck, its long-term persistence may still be threatened by its lack of genetic variability which it may need to meet changing environmental conditions.

Most of the sampled *B. titania* populations have high levels of mean heterozygosity and polymorphism (Table 10). The large geographic distances between sampled *B. titania* populations in this study probably account for the apparent lack of gene flow that is indicated by a large F_{st} estimate and significant genetic heterogeneity at several loci in the sampled colonies.

Historical Biogeography

In addition to providing information concerning the ecological genetics, population structure, and the potential for long-term persistence of *B. improba* group populations allozyme data can be used to test historical biogeographical hypotheses concerning the fate of the ancestral butterfly populations during the late Wisconsin glacial period. Two hypotheses could account for the pattern of distribution and differentiation observed in the *B. improba* group. First, the relict hypothesis posits that ancestral *B. improba* were distributed near glacial margins during the late Pleistocene. As the glaciers receded *B. improba* habitat moved north and upslope. Thus, *B. acrocnema* became isolated first and differentiated enough to be recognized as a full species. More northern populations have been isolated for shorter periods of time and have differentiated only to the subspecies level.

The second hypothesis is a dispersal hypothesis. Ancestral *B. improba* existed in refugia in Alaska during

the last Pleistocene glacial period. As the climate warmed, butterflies dispersed south along an archipelago of alpine habitat, eventually reaching a southern terminus in Colorado. Progressive founder events have lead to the present differentiation of the various species and subspecies on a north-south continuum.

The two hypotheses lead to different predictions about the genetic compositions of the cordilleran populations. Under the dispersal hypothesis, the genetic composition of each population in the cordilleran chain should be an impoverished subset of the one to the north, i.e., there should be a progressive reduction in heterozygosity and in the number of low-frequency and rare alleles in a north-south direction. Under the relict hypothesis, there is no reason to expect declining heterozygosity and nested subsets of alleles in a north-south direction; rather, a much more random pattern of allelic composition and heterozygosity would be expected.

Although there is a progressive loss of heterozygosity from north to south in *B. improba* samples as predicted by the dispersal hypothesis, there are no nested subsets of alleles in the populations sampled along the Cordilleran chain. The southern *B. improba harryi* and *B. acrocnema* samples contain four alleles not found in either the northern or central regions (Figure 8). The trend is similar in southern populations of *B. titania* which have

10 alleles not found in northern and central populations (Figure 8).

On the other hand, the predictions of the relict hypothesis are not supported by the observed distributions of regional alleles either. In both the *B. improba* group and in *B. titania* central region samples have fewer regional alleles than the others (Figures 6 and 8). Random isolation events should result in roughly equivalent numbers of regional alleles. If this were the case, central populations of *B. improba* should have about four regional alleles out of the total of 13 present in all three regions. Similarly, central *B. titania* populations would be expected to have about six of 19 regional alleles under the relict hypothesis. Therefore, the distribution of regional alleles cannot falsify either the dispersal or the relict hypothesis but elements of both hypotheses could have operated in the past to yield the present distribution of butterfly populations.

The results of the allozyme study conform well to the hypothesis that ancestral populations of the *B. improba* group and *B. titania* were isolated by the Wisconsin glaciation in Alaskan refugia and along the margins of the major ice sheets and mountain glaciers to the south. The northern and southern regional samples are genetically similar (Table 9 and Figure 5) reflecting an historical connection between the populations of the two regions.

Most likely, ancestral *B. improba* populations occupied a large and continuous area of North America as the climate cooled during the early stages of the last Wisconsin glaciation. The population was then split into two widely separated parts once the Laurentide and Cordilleran ice sheets coalesced; one segment of the population was confined to Alaskan refugia, and the other existed south of the glacial margins. Both ancestral populations were probably large because genetic drift did not result in much divergence between the northern and southern populations (Table 9 and Figure 5).

The allozyme results for *B. improba* also indicate that colonization of the central region was most likely from the north. Genetic identities between northern and central populations are generally higher than genetic identities between central and southern populations (Table 9). The north and central regions share eight alleles that are not found in the south, while central and southern regions combined do not share a single allele that is not found in the north (Figure 6).

When the distribution of alleles is examined within the central region alone the evidence for northern colonization of the central region is striking. Among the eight loci that are polymorphic in at least one of the central *B. improba* samples (Table 1) the Pink Mountain, BC, population contains six alleles that are not found in the

other two central samples. Among the two more southern samples within the central region Mount Hamell, AB, has one allele not found in the other central populations and Prospect Mountain, AB, has no such alleles. Pink Mountain, BC, and Mount Hamell, AB, share two alleles that are not found in the Prospect Mountain, AB, sample which is the furthest south of the populations in the central region (Figure 1). Only one exception was observed; the two Alberta samples share one allele at the MPI locus that was not observed in the Pink Mountain, BC, sample to the north. There is a similar trend in *B. titania*. Among the 11 loci that are polymorphic in the central samples the more northern Pink Mountain, BC, sample contains 17 alleles that are not found in the more southern Prospect Mountain, AB, sample. The Prospect Mountain, AB, sample has only three alleles that are not segregating in the Pink Mountain, BC, population.

There is a clear trend of declining genetic variability in a southerward direction from Pink Mountain, BC, to Prospect Mountain, AB, (Table 7) among *B. improba* samples within the central region. Successive founder events from north to south are a likely cause of this trend. Furthermore, the fact that the central populations are geographically closer to the northern populations than to the southern populations makes colonization from the north more probable.

Finally, the low levels of genetic variability in the southern *B. improba* group samples (Table 7) can be attributed to recent genetic bottlenecks that occurred when a large and continuous population was gradually confined to the few habitable sites remaining at the end of the Pleistocene. Since *B. titania* is more widely distributed and more of a habitat generalist than *B. improba harryi* or *B. acrocnema* (Ferris *et al.*, 1983; Scott, 1986) it would not have experienced a loss of habitat of the same magnitude. Therefore, *B. titania* would have been able to maintain larger population sizes, which would have ameliorated the loss of genetic variability due to drift in southern *B. titania* populations (Table 10).

Results from the allozyme survey imply that ancestral *B. improba* and *B. titania* populations existed in Alaskan refugia and south of the major ice sheets during the Wisconsin glaciation and that the central Canadian populations were most likely derived from northern populations. Geological and palynological studies can provide an approximate time frame for the deglaciation events that lead to the present distribution of *B. improba* in western North America. By overlaying information about the genetic divergence that has taken place within the *B. improba* group with knowledge of the timing of the geological and climatic events that shaped the present

distribution of these butterflies, further understanding of the rate of evolutionary change can be gained.

Glaciers dominated the landscape of North America for much of the Pleistocene. The last glacial episode, the Wisconsin, began approximately 55,000 years before present (B.P.) when the Cordillaran and Laurentide ice sheets met near the British Columbia and Alberta border in the south and in western Northwest Territories to the north (Flint, 1971; and Reeves, 1973). Although the two ice sheets covered most of northern North America, a large ice-free refugium existed in Alaska and western Yukon Territory with a narrow extension reaching along the border between the Northwest Territories and the Yukon Territory south to near the British Columbia border (Flint, 1971).

Although the ice sheets dominated the North American landscape for thousands of years, they were not static. The Laurentide and Cordilleran glaciers subsided and coalesced several times during the Wisconsin glaciation (Reeves, 1973); thus, the glaciers did not present a constant barrier to migration during this period. Ice-free corridors were open for a few thousand years and then closed as the ice sheets re-expanded. The last glacial maximum that resulted in the closure of the ice-free corridor occurred 18,000 to 20,000 years B.P. (Reeves, 1973; Dyke and Prest, 1987).

There is a rough consensus among investigators as to the approximate time of the opening of the last Wisconsinan ice-free corridor. Mott and Jackson (1982) state that the southern end of the corridor in Alberta was free of ice by 18,000 years B.P. According to Reeves (1973), southern Alberta was ice free by at least 15,000 years B.P. with mountain valleys being deglaciated by 10,500 years B.P. Rutter (1984) indicates that a narrow strip of Alberta from the Jasper-Hinton area south to the U.S. border was not ice covered during the last advance of the Wisconsin glaciers. The Laurentide and Cordilleran ice sheets coalesced north of Hinton, AB, and did not separate until 13,500 to 11,400 years B.P. (Rutter,1984). Finally, Flint (1971) dates the opening of the ice-free corridor at 12,000 to 10,000 years B.P. (Figure 12).

This chronology provides an independent estimate of the length of time the butterflies in the *B. improba* group have had to differentiate. Apparently, the Alaskan refugial populations and the southern glacial margin populations of *B. improba* were separated by ice 18,000 to 20,000 years B.P. Gene flow was certainly curtailed between the two regional groups for 6,000 to 8,000 years until the complete opening of the ice-free corridor approximately 12,000 years B.P. and has probably been minimal ever since given the great distance separating the central and southern regions.

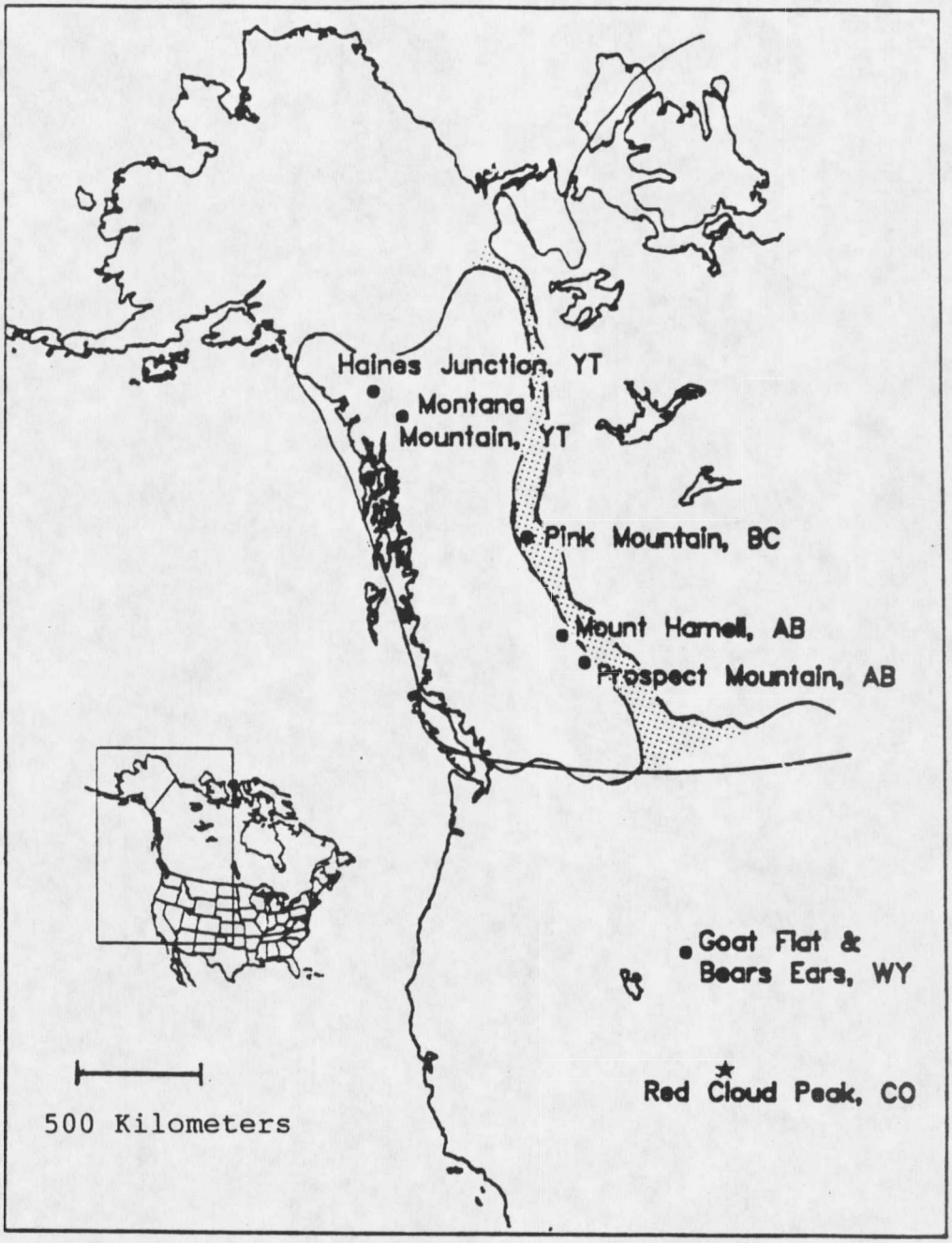

Figure 12. The ice-free corridor in western North America 12,000 to 10,000 years B.P. (after Flint, 1971) and sample sites.

As the ice-free corridor expanded, the butterflies were able to colonize the newly created habitat. Mott and Jackson (1982) analyzed pollen samples from sites within the southern part of the corridor and determined that the environment at the time was similar to modern tundra environments. The proximity of known *B. improba* colony sites in British Columbia and Alberta to the putative glacial fronts of Flint (1971) is illustrated in Figure 12. The present distribution of *B. improba* relative to the glacial fronts strongly implies that the ice-free corridor was the dispersal route used by the butterflies approximately 12,000 years B.P. (Figure 12).

This chronology can be compared to the genetic differentiation present in the *B. improba* group to yield a rough approximation of the rate of evolutionary change in the clade. It is apparent that the nominal speciation of *B. acrocnema* took place within a time span of 18,000 to 20,000 years. Although butterfly populations have existed in the central Canadian region for a shorter period of time they show more divergence from the northern populations than the southern populations do. This pattern of divergence strongly suggests that founder effects were an important factor in the genetic differentiation of these populations. The central *B. improba* populations could have existed for a maximum of 12,000 years.

Further testing of the vicariance/dispersal hypothesis could be carried out in two ways. First, populations to the north of the Haines Junction, YT, and Montana Mountain, YT, populations should be sampled. Samples from this part of the range of *B. improba* are expected to be at least as variable as those already assayed from the Yukon Territory. In addition, all northern region samples are expected to be more genetically similar to one another than to other populations. Second, other means of determining genetic relatedness and variability among the sampled populations should be employed. Analysis of the variability in mtDNA is particularly suited to this investigation. Under the present hypothesis, relationship and variability patterns are expected to be similar in mtDNA to those found in the assayed allozyme loci, if the hypothesis is to be accepted.

Habitat

Field observations led to the hypothesis that *B. acrocnema*, and possibly *B.i. harryi*, have more specialized habitat requirements than other butterflies in the *B. improba* group. Although patches of *S. nivalis* are ubiquitous from tree-line up to near the upper limit of plant growth in the San Juan Mountains, *B. acrocnema* colonies are only known from a very few sites. These sites are at some of the highest elevations which support plant growth and are all NE facing. Apparently, the habitat requirements of the butterfly are more stringent than those of its larval food plant. Northern and central *B. improba* colony sites, in contrast, are generally at mid-slope above tree-line and have a variety of aspects. Although exact data are lacking, northern *B. improba* colony sites appear to be much larger than southern sites. As discussed above, larval feeding ecology and development may interact with host plant phenology in determining the suitability of potential *B. improba* group colony sites.

Studies have shown that, given enough time, isolated populations will diverge both genetically and ecologically from their parental stock (e.g. Carson, 1982; and Grant, 1986). Under the right circumstances impressive radiations can take place over a few million years resulting in

striking morphological and ecological differentiation. The finches of the Galapagos islands are one of many examples of this type of radiation (Grant, 1986). Somewhat more subtle genetic, morphological, and ecological divergences took place over the past 700,000 years or so among *Drosophila* ssp. on the island of Hawaii (Carson, 1982). Therefore, although they have not diverged much genetically from northern populations, one might expect some ecological differences to be detected between disjunct southern butterflies and more continuously distributed northern and central *B. improba*. Because *B. acrocnema* is the most disjunct member of the clade, it has probably been isolated the longest and has diverged enough morphologically to be described as a full species. It is hypothesized that it evolved in allopatry to the point where it differs in habitat requirements from the rest of the *B. improba* group. In addition, given its apparently precarious position in terms of persistence, it may be useful to any future management efforts to know if *B. acrocnema* has diverged ecologically during the 18,000 to 20,000 years that it has been isolated from northern gene flow. Colony site vegetation and physical characteristics are useful parameters in testing this hypothesis because of the discrete nature of the sites and the philopatry of the butterfly.

The loadings on PC1 and PC2 (Table 13) indicate that southern *B. improba* group colonies occur on higher, more north facing, rockier, and less shrub covered sites than central and northern colonies. This corresponds well with personal observations of the sites. The clustering of *B. acrocnema* sites near the means of the two principal components (Figure 9) suggests two conclusions: 1) these sites are more similar to each other than they are to other *B. improba* group sites, and 2) *B. acrocnema* has a smaller ecological amplitude than the rest of the group taken as a whole. Based on this, one would conclude that *B. acrocnema* is somewhat of a habitat specialist requiring a specific kind of habitat within the range of habitats occupied by the entire clade. There are, however, two reasons to be cautious in this conclusion.

First, sites that are geographically closest (i.e. within the same region) are expected to be more similar than more distant sites. Thus, sites in Colorado should cluster together relative to other sites due to environmental autocorrelation. Indeed, sites within all regions tend to form clusters in the principal component space (Figure 9) which suggests that they are more similar with respect to the variables measured than sites in other regions. Because *B. improba* sites essentially surround the *B. acrocnema* cluster in the PCA (Figure 9), however, the

conclusion that *B. acrocnema* occupies a subset of sites occupied by the entire *B. improba* group stands.

Second, there is a biological reason for using caution when interpreting these results. The observed pattern could be an artifact of the assumed metapopulation structure and decline of *B. acrocnema*. The habitat data used in this analysis are from the only five *B. acrocnema* sites known. Presumably they are the best at meeting the needs of the butterfly or are the recipients of migrants from larger colonies nearby. Only the largest colonies at optimal sites would be expected to persist through the present bottleneck. Colonies at marginal sites could have been extirpated by environmental and/or demographic uncertainty, catastrophes, or combinations of these factors. Thus, marginal colony sites would not have been included in the analysis. Furthermore, it is expected that the best sites for the butterfly would have similar characteristics and would, therefore, cluster together in the PCA. Therefore, the only conclusion that can be reached is that under present conditions *B. acrocnema* is probably somewhat of a habitat specialist in comparison to its closest relatives, but this may not necessarily have been the case in the past.

Taxonomy

Gall and Sperling (1980) describe *B. acrocnema* as a previously unknown species of nymphalid butterfly that is closely related to *B. improba*. This conclusion was based on a phenetic analysis of 75 body and wing characters measured from Canadian *B. improba* and *B. acrocnema* from Uncompahgre Peak, CO; *B.i. harryi* specimens were not included in this original analysis (Gall and Sperling, 1980). The designation of *B. acrocnema* as a full species, however, has been disputed by Scott (1986) and others who consider it a subspecies of *B. improba*.

Estimates of genetic similarity derived from electrophoretic data and morphometric data from wing measurements may help clarify the taxonomic status of *B. acrocnema*. Brussard *et al.* (1985) compared estimates of genetic identity (I) between several previously described taxa of nymphalid butterflies and *Drosophila* spp. in order to determine the appropriate taxonomic category for observed estimates of I between pairs of butterfly taxa. It was determined that sibling species of butterflies have, on average, genetic identities of 0.837 while the mean I of butterfly subspecies was estimated at 0.978. There are, however, wide variances around these means which make taxonomic interpretations of these estimates difficult.

Nevertheless, an examination of Figure 9 reveals that all members of the *B. improba* group have genetic identities well above the mean for butterfly sibling species. Thus, although these conclusions should be considered with caution, they indicate that all members of the *B. improba* group could be of the same species, implying that *B. acrocnema* may be better considered a subspecies of *B. improba*.

Morphometric data corroborate this conclusion. The inclusion of *B.i harryi* in the phenetic analysis of wing characters clearly results in a gradual distribution of *B. improba* group individuals along PC2 (Figure 11). The tendency for *B. acrocnema* individuals to cluster in the PCA plot (Figure 11), however, indicates that they are somewhat distinct morphologically relative to *B. bellona* and the other *B. improba* group butterflies. Therefore, electrophoretic analysis and morphometric data both indicate that *B. acrocnema* should probably be considered a well differentiated subspecies of *B. improba* rather than a full species.

SUMMARY AND CONCLUSIONS

Population size estimates and anecdotal evidence indicate that *B. acrocnema* populations have been declining at both known colony sites throughout the 1980's. The butterfly apparently has been extirpated, or nearly so, at its type locality as of 1990. There has been no obvious human caused disturbance or other deterministic factor that could account for the decline. Rather, the decline is hypothesized to be the result of unpredictable environmental events, most notably a series of unusually warm and dry years in SW Colorado, which could have population-wide effects on reproduction and survival. While climatic factors are probably the largest cause of the decline of *B. acrocnema*, other unpredictable factors may also have contributed to the loss of individual populations.

Southern *B. improba* group populations were found to be significantly less genetically variable than northern and central populations. Although there is no direct evidence that this relative lack of genetic variability has affected *B. acrocnema* population persistence, it is expected that long-term survival of any species may be compromised by low levels of polymorphism and heterozygosity. Allozyme results from *B. titania* populations indicate no significant loss of genetic variability to the south. Allozyme results

further indicate that gene flow between, and even within, *B. improba* populations is low. Behavioral studies of *B. acrocnema* collaborate this conclusion (Gall, 1984a).

The apparently low levels of gene flow between *B. improba* group populations and the disjunct distribution of southern populations suggest that Wyoming and Colorado *B. improba* group populations are genetically isolated. Genetic similarity estimates and the distributions of regional alleles suggest that during the last glacial maximum (18,000 to 20,000 years B.P.) the range of ancestral *B. improba* was split into Alaskan refugial populations and southern glacial-margin populations. Subsequent dispersal into an ice-free corridor 12,000 to 10,000 years B.P. from Alaskan refugial populations may have given rise to British Columbia and Alberta populations. Results from *B. titania* support this vicariance/dispersal hypothesis. *B. acrocnema* apparently has had 18,000 to 20,000 years in essential allopatry to diverge from the rest of the clade. The taxonomy of the group is still incompletely resolved, but allozyme and morphometric data suggest that *B. acrocnema* is probably a well differentiated subspecies of *B. improba* rather than a full sister species.

Results from extant *B. acrocnema* colonies indicate that this butterfly has somewhat more stringent habitat requirements than the other members of the *B. improba*

group. *B. acrocnema* colony sites were found to be most similar to *B. i. harryi* sites in Wyoming.

This study yielded several results which may be useful to any future conservation efforts aimed at *B. acrocnema*. By all evidence, this species is in a decline that may take it to extinction. There is little management action that can be taken to ameliorate the effects of environmental stochasticity. Populations that persist during these conditions, however, can be somewhat protected from human caused disturbances, such as collecting and livestock grazing, through the banning of such activities by the federal agencies with jurisdiction over *B. acrocnema* colony sites. Ultimately, listing under the U.S. Endangered Species Act would be the best way to provide protection for the species; this process is currently underway.

Careful monitoring of the known *B. acrocnema* colonies should be done throughout each flight season. A transect method such as that used in the present study would provide relative population size data that could be used to track population trends through each flight season and over several years. This method should be employed for both even- and odd-year broods at Red Cloud Peak, CO. Counts taken on alternate days of each flight season would provide adequate data on population trends at this location. The Uncompahgre Peak, CO, colony site should be visited several times throughout each flight season to ensure that the

presence of even a small number of butterflies can be detected. The other three known *B. acrocnema* locations should also be visited periodically over the course of several flight seasons to determine if they consistently support small numbers of butterflies. Finally, the search should continue for other large *B. acrocnema* colonies in the San Juan Mountains and other nearby ranges in SW Colorado. The vicinity of Baldy Chato, 31 km E of Lake City, CO, should be a priority area for a search because of the presence of several *B. acrocnema* at the site in 1988. Any new colonies should be screened for allozyme variability to provide further information on the population structure of *B. acrocnema*.

Transplantations to re-establish the Uncompahgre Peak colony or to establish new colonies at other sites should not be attempted at this time because this would require the removal of reproductively competent *B. acrocnema* adults from the Red Cloud Peak colony. Such action could be a serious detriment to the already declining Red Cloud Peak population.

Artificial transplantations would be prudent under two sets of circumstances. Firstly, assuming that the decline in *B. acrocnema* numbers is climate related, a return to more normal conditions may allow the recovery of both Red Cloud Peak broods, and potentially the even-year brood at Uncompahgre Peak to early 1980's levels. These naturally

recovered colonies could then be used as sources for transplanted butterflies. In addition, the Uncompahgre Peak odd-year brood could be re-established through leakage (a possibility in light of the allozyme data), or through colonization from another site in an odd year. The latter is unlikely due to the lack of nearby sources of founders and the sedentary nature of *B. acrocnema*. Secondly, other large colonies of *B. acrocnema* may be discovered. These populations could supply individuals for transplantation. In either case, transplants should be planned to ensure the existence of both broods at the type locality and Red Cloud Peak. A number of similar sites should also be selected for the establishment of new colonies to spread the risk of extinction among as many sites as possible.

In the event that *B. acrocnema* is extirpated at both known colony sites and the search for new colonies fails to find any additional ones; the introduction of *B. improba* to former *B. acrocnema* sites might be considered. This should be done only after several years of monitoring at each site has revealed that *B. acrocnema* no longer exists at these locations. The decision to take such action would be expedited by the clarification of the taxonomic status of *B. acrocnema*. Managers will want to know whether an exotic subspecies or species is being considered for introduction into the area. Assuming that this question can be

satisfactorily answered, the source of the introductions would have to be selected.

B. i. harryi from Wyoming are the most similar to *B. acrocnema* both genetically and in terms of habitat. This would appear to make it an ideal candidate for introduction into former *B. acrocnema* colony sites. *B. i. harryi* populations, however, are the least heterozygous of any sampled in the *B. improba* group (Table 7). Initially low levels of genetic variability, in conjunction with a potential founder effect during the introductions, may render transplanted populations extremely depauperate genetically. This could cause inbreeding depression in the short-term and a long-term inability to adapt to the new environment. Neither case is likely to enhance the persistence of the introduced *B. i. harryi* populations.

Northern *B. improba* may be a better choice for introduction into former *B. acrocnema* habitat. The Haines Junction, YT, and Montana Mountain, YT, populations are only slightly less genetically similar to *B. acrocnema* than are *B. i. harryi* populations (Table 9). In addition, these populations are much more genetically variable than are either *B. i. harryi* or *B. acrocnema* populations (Table 7). The additional variability may allow introduced populations to more readily adapt to their new environment even though there are some apparent differences in habitat preference between the two taxa.

Central region *B. improba* are probably the least suitable for introduction into SW Colorado in the event of the demise of *B. acrocnema*. Although they are quite heterozygous (Table 7), these populations are the least similar genetically to *B. acrocnema* (Table 9). Additionally, the habitat of central *B. improba* is considerably different from that of *B. acronema* (Figure 9).

Finally, it should be noted that efforts to re-establish butterfly populations have often been unsuccessful. Pyle *et al.* (1981) point out that several attempted butterfly introductions in North America and England have either failed or have required intensive post-introduction monitoring and management including frequent re-introductions from captive colonies. Thomas (1988), however, notes that some butterfly reintroductions have been successful. In addition, Holdren and Ehrlich (1981) attempted to establish two *Euphydryas gellettii* populations in Colorado with individuals from Wyoming. One transplant apparently persisted for several years while the other was extinct after two years. Given that reintroductions may fail, all reasonable efforts should be made to ensure that the few extant *B. acrocnema* populations persist.

Climate change is probably both the creator and destroyer of *B. acrocnema*. The last Wisconsin glaciation and subsequent warming resulted in a vicariance event that left ancestral *B. acrocnema* isolated near mountaintops in

SW Colorado. The butterfly is rare, a "classic restricted endemic" according to the scheme of Rabinowitz *et al.* (1986); it is narrowly distributed, a habitat specialist, and (was) abundant where found. A relictual distribution, however, does not necessarily make a species prone to extinction. Indeed, *B. acrocnema* probably thrived under these conditions for several thousand years.

The decline of *B. acrocnema* may provide general insights into the extinction process. If recent climate change, or some other environmental perturbation, has pushed *B. acrocnema* to the brink of extinction, it may be useful to identify those traits that made this species vulnerable so that other potentially extinction prone species may be identified. Although the mechanisms are not thoroughly understood, low levels of genetic variability can theoretically affect persistence. The population structure of *B. acrocnema* suggested by allozyme data indicates that inbreeding depression, with its resultant negative effects on survival and fecundity, could be a potential factor in the short-term survival of this declining species. In the long run, low levels of heterozygosity and polymorphism may mean that the species will be unable to adapt to changing environmental conditions such as warmer and drier weather. A metapopulation structure coupled with somewhat specific habitat requirements may result in a decline when climatic

conditions change enough to eliminate occupancy at marginal habitat patches. Populations in marginal habitat patches could provide "stepping stones" for gene flow between patches, thus ameliorating the potential negative effects of inbreeding and the loss of alleles through drift, or act as reservoirs of colonists to unoccupied sites. Both of these potential functions of populations at marginal sites could be important in species persistence.

A final trait that may make *B. acrocnema*, and species like it, vulnerable to extinction is its apparent inability to disperse. Apparently, late season females are the only segment of *B. acrocnema* populations that have any propensity to leave colony sites (Gall, 1984a). The paradox of a metapopulation of nondispersers may be resolved, if environmental change is seen as a subtle form of habitat patch loss. Under good conditions, a few dispersers may have a high probability of finding already occupied sites in which to breed or suitable unoccupied patches in which to establish new populations. In *B. acrocnema* late season females are assumed to carry fertilized eggs which can be laid at new sites to begin new colonies (Gall, 1984a). Under less favorable climatic conditions, the few dispersers that were adequate in maintaining metapopulation structure when habitat patches

were abundant would be unlikely to find suitable habitat patches, occupied or not, and the metapopulation would collapse.

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