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SYSTEMATICS, EVOLUTIONARY BIOLOGY AND POPULATION GENETICS OF THE *CERCYONIS PEGALA* GROUP (LEPIDOPTERA: NYMPHALIDAE: SATYRINAE)

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ABSTRACT.– This study is an overview of the systematics of the *Cercyonis pegala* group of butterflies. Laboratory cultures of three different subspecies were established in the summer of 1993. Interbreeding experiments between different subspecies of *Cercyonis pegala* from Ohio and Colorado were conducted successfully. Breeding of color morphs in the highly variable population from Ohio proved the "form" status of the yellow-banded (*C. p. alope*) and dark-brown (*C. p. nephele*) specimens, instead of subspecific or specific status. Studies of over 5,000 specimens of *Cercyonis pegala* in the major entomological collections, led to the conclusion that Eastern U.S. populations of *C. pegala* have clinal status rather than being separate subspecies. A new treatment is proposed which synonomizes all names of subspecies in the East. The present condition of the systematics of *C. pegala* across the Western United States is discussed, however, the existing extensive use of subspecific designations there is not altered due to insufficient biological and genetic information. This study also attempted to apply analyses of different populations for cuticular hydrocarbons (by gas chromatography) and for genetic variation in genes controlling a number of enzyme systems (via allozyme electrophoresis) for obtaining additional information on the organisms. However, these techniques proved to be not capable of resolving useful variation or differentiating populations or taxa at the subspecific level. Studies of the immature stages of different subspecies of *C. pegala* were also conducted, and these findings led to the conclusion that there are extreme similarities in egg, larval, and pupal characters on the subspecific level in this group. All taxonomically useful characters that have been used to define forms or subspecies in this butterfly complex therefore are confined to the adult stage. Studies on larval biology and mating habits of *Cercyonis pegala* showed that mating is restricted to different hours in the day for different populations, even when all are bred under similar conditions. Also, significant differences were shown in the behavior of larvae of different subspecies. Finally, change in daylength was found to be a significant, if not the only factor involved in breaking the larval diapause, eliminating the usual concept of temperature being the key factor in this process.

KEY WORDS: Acari, allozymes, behavior, biogeography, Canada, *Cercyonis*, diapause, Diptera, distribution, eggs, electrophoresis, evolution, gas chromatography, genetics, Great Basin, hydrocarbons, Hymenoptera, *Hyponephile*, larval biology, *Maniola*, Maniolini, Mexico, *Minois*, Nearctic, North America, Nymphalidae, Orthoptera, pupae.

The butterfly genus *Cercyonis* Scudder is found only in the New World and is confined to North America north of the Tropic of Cancer. Authorities have differed for two centuries in their recognitions of the number of species actually in this Nearctic genus, and much of the confusion in the literature has centered on the "larger" *Cercyonis* associated with the name *C. pegala*, first described by Fabricius in 1775. *Cercyonis pegala* (Fabricius), which is also called by the common name "Wood Nymph," is distributed throughout the United States, northern Mexico, and southern Canada. It presents a complex of subspecies and forms, the recognized number of which varies significantly from publication to publication, depending on the degree of conservativeness of the author. The butterflies are found in a very wide range of habitats: from the dry alkaline deserts of Nevada to the wet meadows of the northeastern states to the subtropical woods of Florida. Populations in different regions and habitats are very distinct in their appearance and biology.

When William H. Edwards (1884) and his contemporaries were describing many of the butterflies in this group during the last century, the representation of this group in collections was rather poor. Besides, the typological concept (see Mayr, 1963) was still dominant in systematics at that time. Therefore, it is not surprising that whenever a new specimen of *C. pegala* from one of the numerous populations of that species group was acquired, it was named as a different species, so different it seemed from the others (Fig. 1-2).

Later in the twentieth century, the pool of distributional and biological knowledge about the butterfly fauna of North America increased significantly. Brown (1964) summarized photographs of Edwards's types and type locality information in one article. He also designated type specimens for many of the taxa and named several new subspecies. Thomas C. Emmel (1969), then a graduate student at Stanford, was able to analyze the whole picture of populations of *Cercyonis* in North America and to review the taxonomic position of all the taxa named to 1968. As a result, the genus *Cercyonis* was split by him into four species: *C. pegala*, *C. oetus* (Boisduval), *C. meadii* (W. H. Edwards), and *C. sthenele* (Boisduval). All species-level taxa of the larger butterflies associated with the *Cercyonis pegala* group were degraded to subspecific category. Some of the former species or subspecies were called genetic "forms" in his summary paper on the genus (Emmel, 1969), however, the promised subsequent

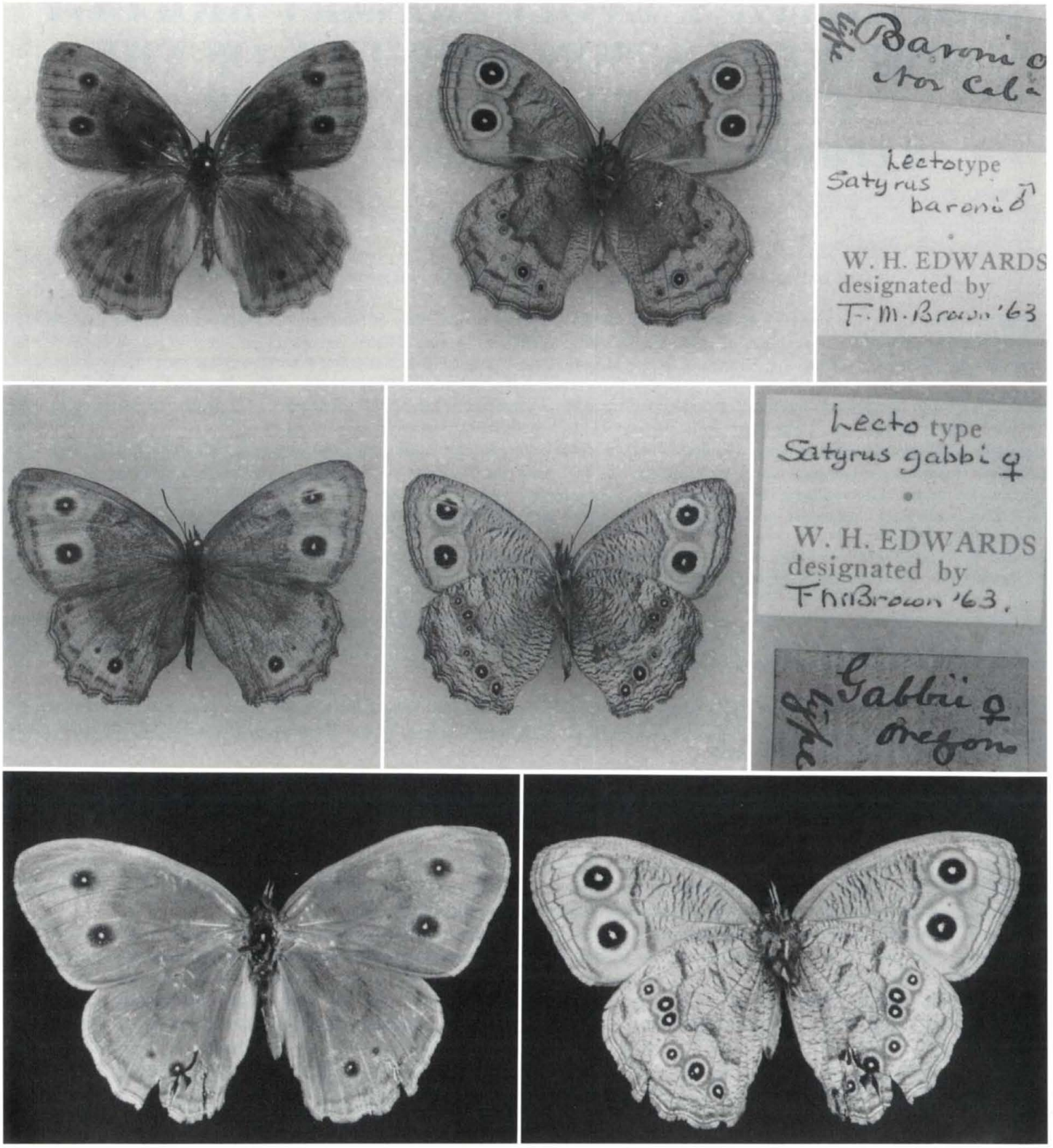


Fig. 1. Type specimens of *Cercyonis pegala* subspecies: (First row) male (upper and under sides) of *C. p. baroni* (W. H. Edwards); (Second row) female (upper and under sides) of *C. p. gabbii* (W. H. Edwards); (Third row) male (upper and under sides) of *C. p. ariane* (Boisduval). Photographed at California Academy of Sciences, San Francisco, Ca., and Carnegie Museum, Pittsburgh, Pa.

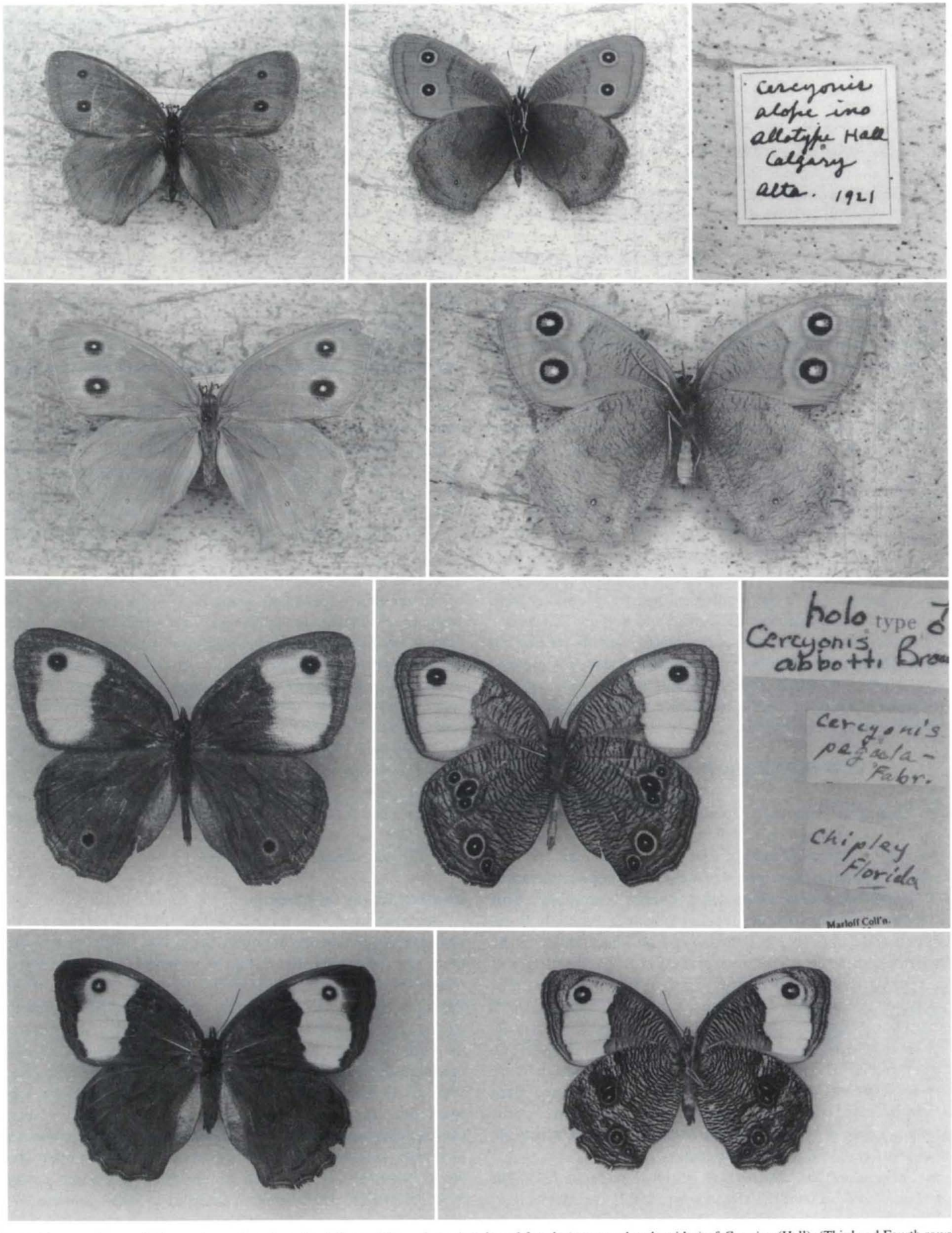


Fig. 2. Type specimens of *Cercyonis pegala* subspecies: (First and Second rows) male and female (upper and under sides) of *C. p. ino* (Hall); (Third and Fourth rows) male and female (upper and under sides) of *C. p. abbotti* (Brown). Photographed at American Museum of Natural History, New York, NY, and Carnegie Museum, Pittsburgh, Pa.

papers, which would explain the detailed treatment of the group, were never published. Emmel's treatment of the genus was used in Howe's edited volume, *The Butterflies of North America* (Emmel, in Howe, 1975) with only some additional biological or ecological details. Subsequent publications, such as the *Catalog/Checklist of Butterflies of America North of Mexico* (Miller and Brown, 1981), followed Emmel's system of recognizing four basic species in the genus and treating many of the older taxa as subspecies. Instead of the seven *C. pegala* subspecies of Emmel, however, the Miller-Brown catalog recognized 13 subspecies, only one of these having been described after Emmel's revision (Emmel and Mattoon, 1972). After the Miller-Brown publication, no new taxa of *Cercyonis* were described until George T. Austin (1992) reviewed the *Cercyonis pegala* populations of the Great Basin and named six new subspecies from different river-valley drainages. He also indicated that the group's taxonomy is a mess and requires thorough revision.

The wide geographic variability and the unstable systematic treatment of the *Cercyonis pegala* complex of satyrine butterflies attracted my interest. To resolve some of the questions about this group, I initiated a multifaceted approach, involving traditional morphological and wing-pattern examinations of adults in museum collections combined with procuring live material from diverse populations and crossing these in wide geographic crosses to examine genetic compatability and the behavior of adult and larval characters in "hybrid" offspring. My research goals were to (1) review the systematics and possible evolution of the group from the perspective of the typological approach utilizing adult characters; (2) compare the life history and biology of western, eastern, and southern members of this highly variable species; (3) analyze variation in cuticular hydrocarbons and allozyme proteins to see if these molecular approaches might shed some light on evolutionary divergence and relationships in this complex group of North American butterflies.

SYSTEMATICS AND EVOLUTION

The Typological Approach Utilizing Adult Characters

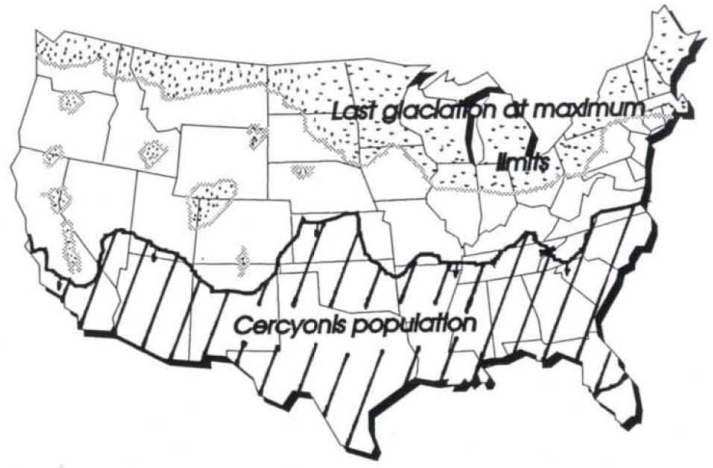
In 1993-94, I undertook trips to major U.S. museums and photographed type specimens of *Cercyonis pegala* taxa. A more essential result from these trips was that I could inspect thousands of *C. pegala* specimens accumulated in these institutions. That allowed me to understand the biogeography of *C. pegala* in North America and come to the conclusion (see Discussion) of the clinal rather than subspecific nature of different *C. pegala* taxa in the eastern parts of the United States. Where western U.S. populations are concerned, the representation of material is still rather poor in museum collections. However, my own modest experience of collecting in the West, combined with that of T. C. Emmel and G. T. Austin, suggests a higher and much more frequent degree of isolation of populations there in much more diverse habitats, which has led to quicker radiation. This radiation, however, is probably far from the stage at which the populations could be called subspecies.

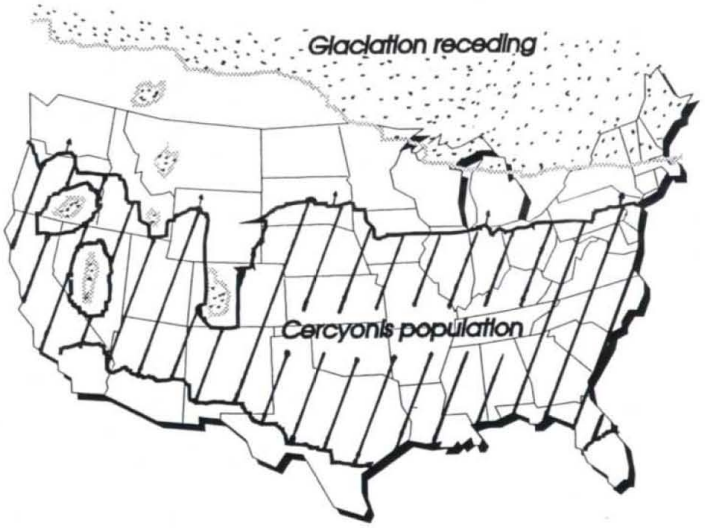
In many cases the distinctness of local populations might correlate with the Founder Effect (Mayr, 1963), as my breeding experiments suggest (see below). That might be the case with *C. p. wheeleri* (W. H. Edwards), an unusual phenotype described only from one location at Owens Lake, east-central California. It is mainly characterized by an invariably double apical ocellus on the forewing. No other *C. pegala* population expresses the regular appearance of this character; however, it can be found occasionally in all adequately sampled (>100) *C. pegala* populations (Fig. 18). If we hypothesize that one of those unusual females started the *C. p. wheeleri* population, the appearance of this "subspecies" is easy to explain. It would also become possible to explain why this population has gone extinct after the turn of the century after its initial discovery by the Wheeler Expedition of 1873 (Brown, 1955, 1956). More recent attempts to obtain new specimens of this taxon have failed (Comstock, 1927; Emmel, 1969): the population, so unusual and so small and isolated from others, could not have been very stable in an evolutionary sense.

Some other characters besides forewing ocelli are used by Austin (1992) to describe Great Basin "subspecies," but these traits appear to be quite variable within populations that I have cultured. Take, for example, the appearance of the number of dorsal hindwing eyespots. This character is used by Austin (1992) to differentiate all of the Great Basin taxa. The average number of these eyespots in each of the populations is put forth as one of the defining characters of each *C. pegala* subspecies. However, to take but one example, I raised 30 specimens which were F1 offspring of a single female from the Ohio population; all had three or four eyespots on the dorsal side of the hindwing. The rest of the females from that population produced F1 offspring with one regular eyespot. If the first female had founded an isolated new population with solely its production of eggs at that site, it would have created a local population with a unique spotting phenotype. But in no way should this unique brood and subsequent colony be called a "subspecies." Subspecific differences, like species differences, are a combination of several characters, not just one character, as was correctly stated by Remington (1950). The study of long-term microevolution in local populations of *Cercyonis oetus* in Colorado conducted by Thomas Emmel suggests that even populations of neighboring meadows have unique mean spotting patterns, which have proved to be stable in some cases during the whole period of study of over 30 years (Emmel, unpublished).

Possible Scheme of Evolution

The position of *Cercyonis* Scudder (1888) as a genus on the phylogenetic tree of the Satyrinae is not very clear. There seem to be no close relatives in the New World. Superficially, it resembles *Minois dryas* (Scopoli), the only member of the Palearctic genus *Minois* Hübner, which is found in Europe, Russian Far East and Japan. Miller (1968), however, suggests on the basis of wing venation, antenna, and leg structure, that this resemblance is superficial. He places *Cercyonis* next to the genus *Maniola* Schrank of Europe in the otherwise Palearctic tribe Maniolini. In another paper (Miller and Emmel, 1971), Miller moves South American satyrids, described by different authors as *Cercyonis*, into new genera, stating unrelatedness of those to true North American *Cercyonis* and moving those genera into another tribe. However, no explanation exists as to when or where any intermediate steps of "*Maniola*"-*Cercyonis* evolution in North America disappeared.



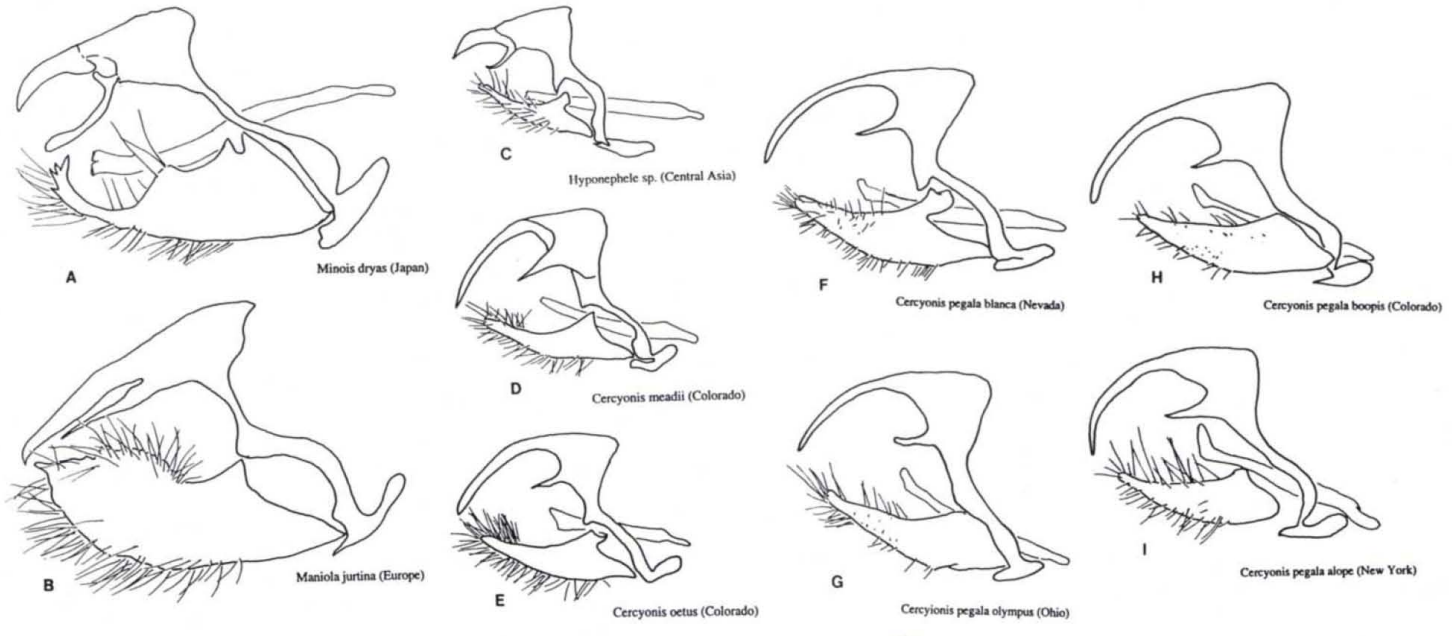


In any case, members of the *Cercyonis* group seem to be very closely related and very recently evolved. Practically no differences in genitalia or other sclerotized structures are found among the different species of *Cercyonis* (Sourakov, personal observation) (Fig. 4-5), whereas the Palearctic *Maniola* shows such differences on the subspecific level (Higgins, 1975). The immature stages of all the *Cercyonis* are also quite similar, and mostly differ in the coloration pattern of mature larvae rather than spination or other structural characters (Fig. 13). Genitalic structures (Fig. 4) and immature stages of *Minois* and *Maniola* confirm the suppositions of Miller. Even though adults of *Minois* very closely resemble *Cercyonis* in color pattern, the two genera are quite different in these characters.

The possible scheme of evolution of such a diversity pattern as the one found in *Cercyonis* was well described by Remington (1950). He tied the subspeciation process for many North American Lepidoptera to the end of the last Pleistocene glaciation. Applying part of this scheme could therefore suggest the following scenario for evolution of the genus *Cercyonis*. The Arctic ancestor or ancestors of *Cercyonis* moved south across North America preceding the accumulating masses of glacial ice. When the climate became warmer and the ice zone started retreating northwards, our *Cercyonis* followed it north, leaving the most warmth-adapted settlers behind (Fig. 3). Those probably became today's southern *C. pegala* populations. Smaller and darker individuals, better adapted to the cold climate, continued north, or climbed the mountains, where the climate still approximated that of the far north. These mountain populations were

Fig. 3. The glacial movements of the last Pleistocene glaciation, and corresponding movement of a hypothetical butterfly species across the North American continent (after Remington, 1950).

Fig. 4. Sclerotized male genitalic structures of *C. pegala* and related taxa: (A) *Minois dryas* (Japan); (B) *Maniola jurtina* (Europe); (C) *Hyponephele* sp. (C. Asia); (D) *Cercyonis meadii* (Colorado); (E) *Cercyonis oetus* (Colorado); (F) *Cercyonis pegala blanca* (Nevada); (G) *C. p. olympus* (Ohio); (H) *C. p. boopis* (Colorado); (I) *C. p. alope* (New York).



isolated from each other by warmer valleys and intrinsic physiological differences in temperature tolerance. They must have produced species and subspecies of presently existing mountain species, such as *C. sthenele, C. oetus* and *C. meadii* (Fig. 7).

While carrying out my breeding experiments, I discovered that *C. pegala* larvae could skip their normal sixth instar and pupate at the end of the fifth instar. That happened under unusually cold (for *Cercyonis*) conditions in laboratory broods cultured in November 1993. All of the specimens produced fall into significantly smaller size limits (Fig. 17). This observation gives me the latitude to speculate that the switching from six to five larval instars could have played a saltational role in the speciation process during the last Pleistocene glaciation, when higher-elevation *Cercyonis* populations subject to cooler temperatures may have given rise to the other species of *Cercyonis*, all of which have five larval instars and are smaller than *Cercyonis pegala*. All three of these species are found in patchy populations and occupy different elevations in the Rocky Mountains and/or Pacific ranges, although occasionally two or three are sympatric. *Cercyonis pegàla* must have left scattered populations in numerous valleys between the Rockies and the Pacific Cascade Ranges, which, being highly isolated, formed all those numerous "subspecies" or ecological races seen there today. In many of those localities, populations might have gone extinct and then been founded again many times, or were populated much later than others to the south. Regular extinction and repopulation of local populations was observed in the study of *C. oetus* by Emmel (unpublished), and occurs regularly and naturally in other well-studied butterfly species (e.g., Ehrlich *et al.*, 1975). Further north and to the east, smaller and darker individuals continued invading new territories. The newly acquired characters undoubtedly both proved to be important in thermoregulation, allowing butterflies to warm up quicker and keep the heat longer in the early morning hours, and thus allowing more of the most valuable daylight hours to be available for nectaring and courting.

Distribution and Taxonomy

As noted above, there are currently four generally recognized species in the genus *Cercyonis*. Three species are restricted to the higher mountains and Great Basin Desert or woodland areas and inland ranges of the Pacific Coast states inland to the Great Basin (*C. oetus, C. meadii,* and *C. sthenele*). Only the larger one, *C. pegala*, is distributed across the North American continent (Fig. 7). The *C. pegala*-related populations are found from sea level up to elevation about 7000 ft. (2300m). The distribution of the named subspecies is much harder to describe. There is substantial confusion in the literature and among lepidopterists with regard to subspecific names, not only because several names were often applied to the same phenotype, but also because many populations consist of individuals resembling several different "subspecies". Besides, the fact that every local population has a unique average phenotype makes people wonder why a particular population is assigned to this or that nominotypical name, when it is obviously different from the phenotype found at the type locality. Unfortunately, sometimes people move from wondering to action, as happened to George Austin, who described six new subspecies from the Great Basin area (Austin, 1992). Hence a great many specific or subspecific names have been proposed for the larger *Cercyonis* in North America.

According to the more conservative revision of Emmel (1969), the distribution of *C. pegala* and its named subdivisions looks as follows:

***Cercyonis pegala pegala* (Fabricius)** is distributed from the Mississippi Valley east to the Atlantic Coast and from the Gulf States to North Carolina and New Jersey.

***Cercyonis pegala alope* (Fabricius)** ranges from Virginia and New Jersey, north to eastern Quebec and Maine, and into New York northward and westward, the yellow-patched *C. p. alope* integrates with the completely dark *C. p. nephele* (W. Kirby), and also with the somewhat lighter *C. p. ochracea* (F. & R. Chermock) (in Ohio). *Cercyonis p. alope* and *C. p. nephele* populations are often parapatric (Shapiro, 1974). In Miller and Brown's *Catalog/Checklist of North American Butterflies* (1981), following Emmel (1969), these forms are treated as subspecies.

***Cercyonis pegala carolina* (F. & R. Chermock)**, to the south, blends with *C. p. alope*.

***Cercyonis pegala maritima* (W. H. Edwards)**, an unusual darker-yellow form, is found at eastern coastal points from Massachusetts to Virginia. First individuals, emerging in the Piedmont are of Virginia, are called *C. p. maritima*, while those that emerge later in the season are called *C. p. alope* (Clark, 1951).

***Cercyonis pegala texana* (W. H. Edwards)** ranges from central Texas north to Kansas and Missouri.

***Cercyonis pegala boopis* (Behr)** ranges from central New Mexico and Arizona north through Colorado to South Dakota and west to the Pacific Coast, where it is distributed from Central California north to British Columbia on the coastal side of the Cascades and the Sierra Nevada. Emmel (1969) mentions that there are many different local forms of *C. pegala* in that region, but mercifully does not name them. Two of these Pacific forms were named by earlier authors as "*incana*" (W. H. Edwards) and "*baroni*" (W. H. Edwards).

***Cercyonis pegala ariane* (Boisduval)** occurs in lowland areas of Utah, Nevada, eastern California, eastern Oregon, and eastern Washington. The names "*gabbii*" (W. H. Edwards), "*wheeleri*" (W. H. Edwards), and "*stephensi*" (W. G. Wright), were also applied to these populations generally referred to as *C. p. ariane* in current butterfly literature.

***Cercyonis pegala damei* (Barnes & Benjamin)**, an unusual red-flushed population, occurs on the North Rim and northern slopes of the Grand Canyon in Arizona. Emmel (1969) thought originally that *C. p. damei* might have represented the result of natural hybridization and back-crossing with introversion of wing characters from another species, *Cercyonis meadii* (W. H. Edwards), into the sympatric *C. p. boopis* population. However, subsequent study of adults by Emmel (unpublished) suggests that it represents a hybridization zone between *Cercyonis sthenele masoni* (Boisduval), which also occurs toward the bottom of the Grand Canyon, and *C. meadii* along the Rim. When compared by me in the present study, the larvae of *C. p. damei* also show characters typical of *C. sthenele* (Boisduval) populations.

In general, Emmel (1969) in his revision does not state firmly the taxonomic status of one or another population, applying the term "form" to most of them, but not formally synonymizing their specific or subspecific names. F. Martin Brown (who was

primarily responsible for the *Cercyonis* arrangement; in litt. to T. C. Emmel) and Lee D. Miller largely followed Emmel (1969) in their subsequent catalog (Miller and Brown, 1981), but did not provide any additional information to support their often different use of "form" versus "subspecies" status for various names. The taxonomic treatment of *C. pegala* proposed by Emmel (1969) is compared below with the listing from the Miller and Brown (1981) catalog (modified to compare with the Emmel list):

Emmel's system, 1969:

Cercyonis pegala (Fabricius, 1775)
- a. *pegala pegala* (Fabricius, 1775)
- b. *pegala alope* (Fabricius ,1793)
  - f. *nephele* (Kirby, 1837)
  - f. *maritima* (W. H. Edwards, 1880)
  - f. *ochracea* (Chermock & Chermock, 1942)
  - f. *carolina* (Chermock & Chermock, 1942)
- c. *pegala texana* (W. H. Edwards,1880)
- d. *pegala ino* (Hall, 1924)
- e. *pegala boopis* (Behr, 1864)
  - = *olympus* (W. H. Edwards, 1880)
  - = *borealis* (F. H. Chermock, 1929)
  - f. *baroni* (W. H. Edwards, 1880)
  - f. *incana* (W. H. Edwards, 1880)
- f. *pegala ariane* (Boisduval, 1852)
  - f. *wheeleri* (W. H. Edwards, 1873)
  - = *hoffmani* (Strecker, 1873)
  - f. *gabbii* (W. H. Edwards, 1870)
  - f. *stephensi* [♀] (W. G. Wright, 1905)
- g. *pegala damei* (Barnes & Benjamin, 1926)

Miller and Brown's system 1981:

Cercyonis pegala (Fabricius, 1775)
- a. *pegala pegala* (Fabricius, 1775)
  - = *maritima* (W. H. Edwards, 1880)
- b. *pegala nephele* (Kirby, 1837)
- c. *pegala alope* (Fabricius, 1793)
  - = *ochracea* (Chermock & Chermock, 1942)
  - = *carolina* (Chermock & Chermock, 1942)
- d. *pegala texana* (W. H. Edwards, 1880)
- e. *pegala ino* (Hall, 1924)
- f. *pegala boopis* (Behr, 1864)
  - = *baroni* (W. H. Edwards, 1880)
  - = *incana* (W. H. Edwards, 1880)
- g. *olympus* (W. H. Edwards, 1880)
  - = *borealis* (F. H. Chermock, 1929)
- h. *pegala ariane* (Boisduval, 1852)
  - = *gabbii* (W. H. Edwards, 1870)
- i. *wheeleri* (W. H. Edwards, 1873)
  - = *hoffmani* (Strecker, 1873)
- j. *stephensi* [♀] (W. G. Wright, 1905)
- k. *pegala damei* (Barnes & Benjamin, 1926)
- l. *pegala blanca* (Emmel & Mattoon, 1972)
- m. *pegala abbotti* (F. M. Brown, 1969)

Further additions to the *C. pegala* taxonomic puzzle were made by Austin (1992), who revived several previously sunk subspecific names and introduced six new ones for different populations located within the Great Basin of Nevada and adjacent states, all named from various river drainages: *C. p. gabbii*, *C. p. stephensi*, and *C. p. wheeleri* were elevated to the status of formal subspecies; *C. p. paucilineatus* Austin, *C. p. utahensis* Austin, *C. p. carsonensis* Austin, *C. p. pluvialis* Austin, *C. p. walkerensis* Austin, and *C. p. paludum* Austin. Austin (1992) based his descriptions of these six new subspecies on characters which, as will be shown below, hardly can be considered subspecifically diagnostic, because they are quite variable within every one of the subspecies. He utilized an eclectic mixture of phenetic and typological approaches rather than taking the approach of evolutionary biology, which would recognize the variation of characters and would be more appropriate in dealing with so young and dynamic a group. Nevertheless, he deserves credit for sampling and analyzing unknown and remote populations of Nevada, Utah, and California, and for pointing out their uniqueness.

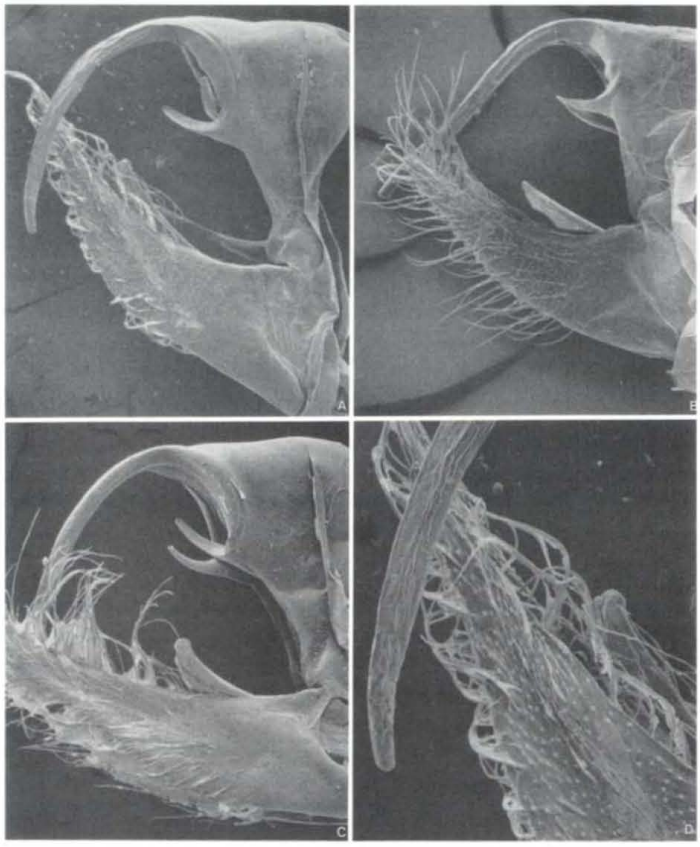


Fig. 5. Micrographs of male *Cercyonis* genitalia: (A) *C. pegala* from Colorado (40x); (B) *C. oetus* (50x); (C) *C. pegala* from Florida (30x); (D) *C. pegala* from Colorado (100x).

System Proposed

Defining types of subspecies in the situation described for the eastern United States might be useful only if, following the method proposed by J. S. Huxley (1940), one referred to intermediate populations as a cline, followed by the names of both subspecies hyphenated. The question would arise, however, which subspecific names to use. Besides, there are problems with local populations which have developed similar phenotypes independently. For example, the name "*nephele*", which is

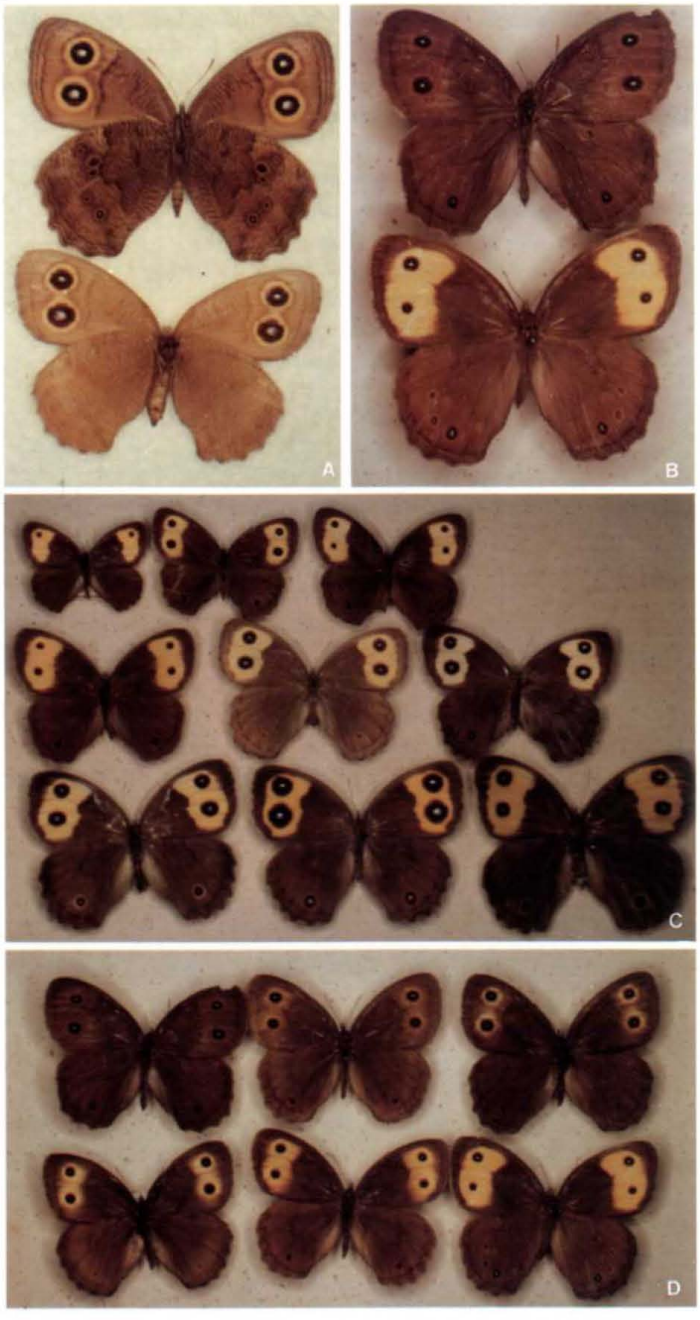


Fig. 6. Clinal and discrete variation in *Cercyonis pegala* in the eastern United States: (A) dorsal eyespots are often used as a character to distinguish subspecies; however, in many unconnected populations this character is extreamly variable (shown are two individuals from the same local population in eastern Canada); (B) "*alope*" and "*nephele*" forms are often parapatric and found in monomorphic populations (many populations contain intermediate forms); (C) size of *C. pegala* throughout the eastern United States is quite variable and has often served as a taxonomic character to delineate subspecies (one might notice the clinal nature of such variation by studying thousands of specimens in the major museum collections); (D) offspring of a single female from the Ohio population.

applied to typical dark populations in the northeastern U.S. was also assigned to the dark Great Basin populations by Scott (1986). The whitish "*blanca*" Emmel and Mattoon (1972) resembles other whitish populations subsequently assigned by Austin (1992) to a number of *C. pegala* subspecies, such as *C. p. stephensi* and *C. p. utahensis*. One could continue by linking under various names other different lighter-colored, stronger-striated, spotless, or heavily spotted, etc., populations scattered all over the United States.

Such a situation is not defensible taxonomically. In a merely practical sense, when does one stop naming new subspecies? To avoid confusion, I would propose to apply only two names in the eastern part of the United States: "*pegala*", referring to yellow-patched populations and "*nephele*", referring to all-dark populations (Fig. 6). Eventhough those populations are found parapatrically and hybridize along the northeastern-central suture zone (Remington, 1968), and could have possibly been isolated in the past, they should bear the status of ecotypes rather than subspecies (see discussion below). The situation in the *C. pegala* of the western United States seems to be more complex, and to be properly interpreted, must await the review of an evolutionary biologist rather than a systematist. I can see, however, how my proposition might be unpopular among authors of faunistic works, who like to have one or more endemic subspecies in their backyard to enhance the attractiveness of their work or geographic area to prospective collectors.

I am not supportive of naming isolated Great Basin or other *C. pegala* populations. There are two major reasons. First, if there are six populations named from a relatively small area in the Great Basin, as was done by Austin (1992), then there is no reason not to name hundreds of other statistically different populations across the Rockies to the West Coast. Such a scenario would lead to a much greater "taxonomic nightmare" than the one (a scattering of various populations having different spotting averages) presented by Austin. Second, the taxonomic status of the individuals that phenotypically belong to one subspecies, but actually are found within the population of another subspecies, becomes unclear. If they were immigrants, they could be considered as temporary invaders from one subspecies into the geographic zone of another. However, in most of these cases, the phenotypes are consistently present in low numbers in all populations and probably present a case of balanced polymorphism of the basic spotting-pattern genes and their alleles.

The following listing summarizes a possible tentative classification of *C. pegala* populations with only three subspecies, although more study is needed for the western forms:

Cercyonis pegala (F.)

a) *C. pegala pegala* (F.) — eastern North America
(= *abbotti*, *alope*, *borealis*, *carolina*, *ino*, *maritima*, *nephele*, *ochracea*, *olympus*, *texana*)

b) *C. pegala boopis* (Behr) — Rockies to Cascades; California
(= *baroni*, *incana*)

c) *C. pegala ariane* (Bdv.) — Great Basin
(= *blanca*, *carsonensis*, *gabbii*, *hoffmani*, *paludum*, *paucilineatus*, *pluvialis*, *stephensi*, *utahensis*, *walkerensis*, *wheeleri*)
[*damei* to synonymy of *C. sthenele*].

Discussion

A subspecies may be defined as a taxonomically recognized aggregate of local populations of a species inhabiting a geographic subdivision of the range of the species (see also Mayr, 1963). Already in the middle of this century, it was generally recognized that the better the geographic variation of a species is known, the more difficult it becomes to delimit subspecies. Wilson and Brown (1953) have pointed out four characteristics of geographic variation which contribute to these difficulties: (1) the tendency

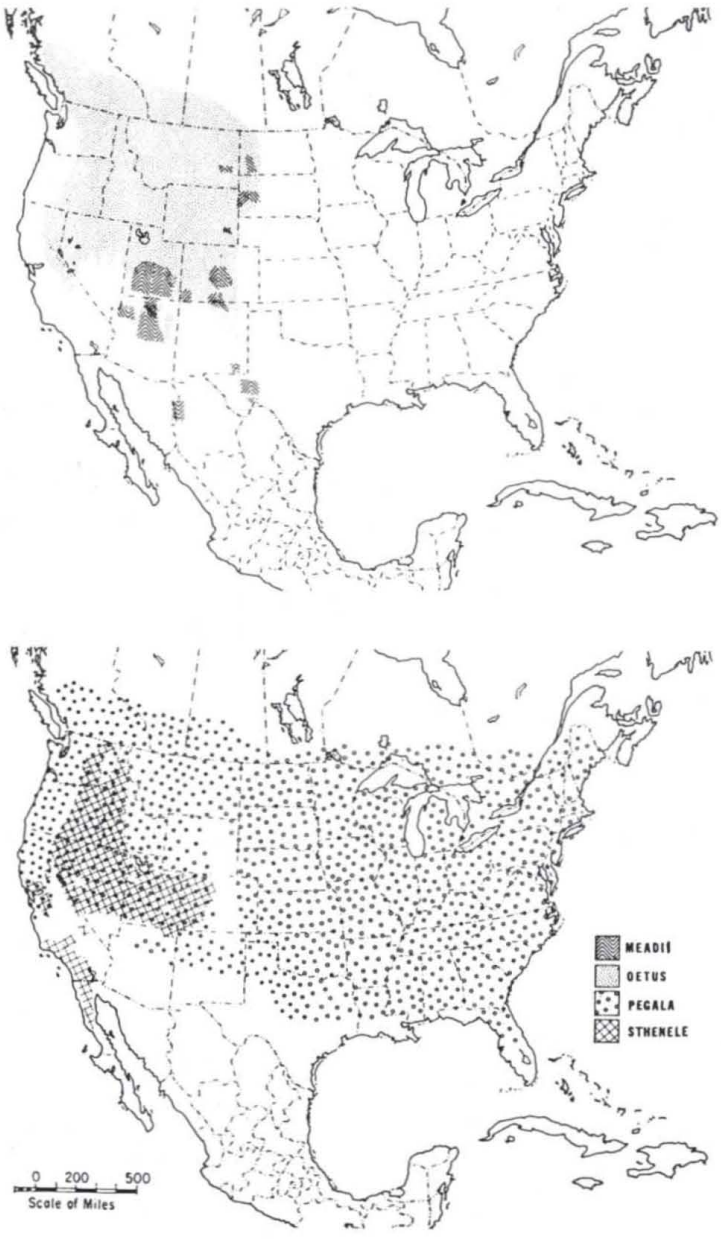


Fig. 7. Distribution of the four species of *Cercyonis* in North America (after Emmel, 1969).

of different characters to show independent trends of geographic variation, (2) the independent reoccurrence of similar phenotypes in widely separated areas, (3) the occurrence of microgeographic races within formally recognized subspecies, and (4) arbitrariness of the degree of distinction used sometimes for subspecific separation of slightly different local populations.

Mayr (1963) emphasizes how some taxonomists have misused the subspecies category in their enthusiasm to name new taxa or describe geographic variation in a species:

> "This definition (of subspecies as individuals that conform to the type of the subspecies) induced many authors to compare carefully material from every newly established locality with specimens from the type locality of a previously described subspecies. Whenever a thorough biometric-morphological analysis established a mean difference between the samples, this was considered sufficient justification by these authors to describe a new subspecies. In the more intensively studied groups of animals this approach has led to a wild-goose chase for new subspecies, and has seriously impaired the usefulness of subspecies category."

Further, he adds several important qualifying points to the subspecies definition stated above: (1) the subspecies is a collective category (it consists of slightly different populations); (2) subspecies should differ taxonomically (not statistically), i.e., they should contain diagnostic morphological characters; (3) it could be impossible to assign every particular individual to a subspecies because of variability, but it should be possible to do so with populations; (4) each subspecies inhabits a certain part of the species' range; it can be polytypic (discontinuously variable). He also stresses the lack of relationship between the subspecies category (a product of isolation) and the cline (a product of the combined interaction of environmental adaptation on one side and genetic flow on the other).

The first point allows for even the most radical taxonomist to show some tolerance to the unnamed morphologically distinct populations. How much tolerance to allow, I think, should depend on the degree of differences, their variability, and possibly, even number of subspecies which would come out of a more-or-less liberal revision (one does not want to deal with hundreds of subspecific names, just because of the inconvenience).

The second point emphasizes the necessity to have characters that would allow one to identify most (say, 95%) of the individuals of the population as belonging to one subspecies. If there are populations in which more than 5% of individuals consistently express a distinctive character state of another subspecies, those subspecies should be synonymized and populations that differ in this character only should be assigned the same suspecific name. However, the specimens bearing one or another character state could be still assigned to the one or another form; accordingly, the names used as subspecific taxa would be used as form names. The value of having a form name would be in the recognition of the existing differences without damaging the subspecific category as a useful evolutionary (phylogenetic) unit.

The third point, from my perspective, states that there should not be any population that does not belong to one or another subspecies. If we deal with a clinal variation of the character, the whole group of populations involved should be assigned to one or another subspecies, providing that there are no other characters which could be used for supporting the subspecific status of the populations. Point three also states the possibility of finding occasional phenotypes of one subspecies in the area of another. A 5% tolerance level is chosen by me arbitrarily, but it is also used in biological statistics extensively.

I would interpret the fourth statement by Mayr (1963) as follows: no subspecies can be found in two areas separated by the area occupied by another subspecies. From my point of view, this situation would contradict the valuable phylogenetic approach to taxonomy by which all groups that are recognized taxonomically should be monophyletic. But there can be a situation where two populations independently evolved to similar phenotypes, under the influence of similar environmental factors. Let us look at a hypothetical example of two separate valleys with a mountain in the center. If there are similar melanic populations on top of those mountains, which meet all the criteria of subspecies, they

could not be assigned the same subspecific name because they are not monophyletic but instead are expressing independently evolved homoplastic characters. I am using this example because it is not unusual to find melanic populations of different species at higher elevations (a condition that is usually interpreted as being the result of selection for better thermoregulatory ability to absorb solar radiation while basking). Of course, populations belonging to the same subspecies could live in isolated existence and be recognized under that one subspecific name as long as there is evidence that they belong to a monophyletic group, or rather when there is no evidence to prove the opposite.

All that has been said in the preceding paragraphs about the difficulties of the application of the subspecies category can be applied to the case of *C. pegala*. It is getting even more difficult to make taxonomically correct decisions in this and many other butterfly groups with the disappearance of habitats and loss of intermediate populations and phenotypes. In the eastern United States, the problem could be solved in *C. pegala*, as I mentioned earlier, by stating the clinal nature of all the populations. In the western United States, *C. pegala* populations, it could be resolved by use of the reasonable doubt when thinking about creating a new name. Without some moderation on the part of systematists, unlimited definition of subspecies becomes a practice which only will confuse the prospective users of the systematists' work, when so many subspecies are named from local forms.

FIELD OBSERVATIONS AND BREEDING EXPERIMENTS

Materials and Methods

In summer 1993, I obtained females from three populations of *C. pegala*: from Fruitland Mesa, Mesa Co., Colorado; from the Ohio Turnpike (I-80), near Exit 30, Ohio; and from Gainesville, Alachua Co., Florida. I also had a chance to sample populations from Siskiyou Co., California, and from Rock Creek Canyon, El Paso Co., Colorado. For each population, data on the number and characteristics of eyespots on the wings and on the wingspan were recorded. A code of four numbers was used to express the degree of development of each eyespot: "0" stands for absent eyespot, "1" for simple dark spot; "2" for a dark spot with external lighter ring present; and "3" for a fully developed eyespot with an external tan ring and a white "pupil" area in the center of the black spot of the ocellus. The size of the wingspan was measured with a digital micrometer accurate to 0.01mm and represents the maximum length of the left forewing along the costal vein from the body to the apical tip.

Eggs (Fig. 8) were obtained from three populations of *Cercyonis* by placing live females into pint-sized, white cardboard ice-cream cartons covered with netting, with some dried grass blades and stems in the bottom. The presence of grass does stimulate a female to lay eggs, but it is not essential. Cartons were kept in the shade, but with sufficient indirect light to produce flight or walking activity. Direct sunlight proved to be lethal, butterflies overheating within 5-10 minutes. Females were fed with a 25% sugar solution every day. They began laying eggs approximately a week from the day of emergence and mating (a virgin female was usually mated to a male on her first day after emergence). A single female can lay 300-400 eggs in 30 days. The first instar larvae (Fig. 12B) hatch within a week and do not feed in nature until the following year. In the lab, I simulated diapause conditions by placing the first instar larvae at 5°C for a week and then transferring them into a freezer at -5°C. The exposure to freezing needed to be not less than 30 days and needed to be followed by a week of adaptation at 5°C to break diapause successfully. Then the larvae were transferred onto pots with a fresh (10 day old) growth of Kentucky bluegrass (*Poa pratensis*) (Gramineae) and allowed to grow (Fig. 15).

Larval Morphology

I examined larvae of the populations I kept in culture. No differences were found between either the first instar stages or the last instar larvae. All of the *C. pegala* subspecies I have reared and examined have six larval instars. I found, however, that the number of instars is not firmly fixed. In measuring the size of the larval head capsule of the "last" instar larvae prior to pupation with the first bred generation, I discovered that those larvae only went through five instars prior to pupation and all the adults were much smaller than the natural size range (Fig. 17). In the thorough description of immature stages of *C. p. blanca*, Emmel and Mattoon (1972) state that this subspecies has only 5 larval instars.

In addition, on the integument of the mature larvae, I found many straight setae with a crown-shaped apex, as well as many mushroom-shaped setae (Fig. 10-11). For reasons unknown to me, these kinds of setae were not noted by previous observers of *C. pegala* life histories (e.g., Edwards, 1884) nor were they mentioned in the description by Emmel and Mattoon (1972). The differences in *C. pegala* larval coloration that were noted even by Edwards (1884), and assigned by him to represent subspecific characters, seem actually to be partly genetic variation between individuals and partly maturational variation dependent on the time passed since last molting (Fig. 14). The differences between larvae of different species of *Cercyonis* seem to be restricted to coloration and pattern (Fig. 13).

Pupae in all three populations were grass green. However, black-and-white marked forms would appear occasionally in Colorado populations (Fig. 14).

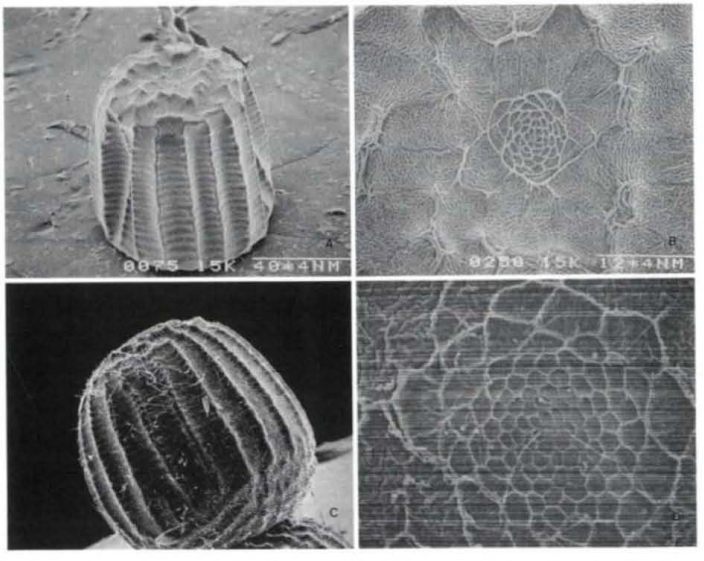


Fig. 8. Micrographs of *Cercyonis* eggs, lateral view and micropylar (dorsal) view: (A-B) *C. oetus* from Florissant, Teller Co., Colorado; (C-D) *C. pegala* from Gainesville, Alachua Co., Florida.

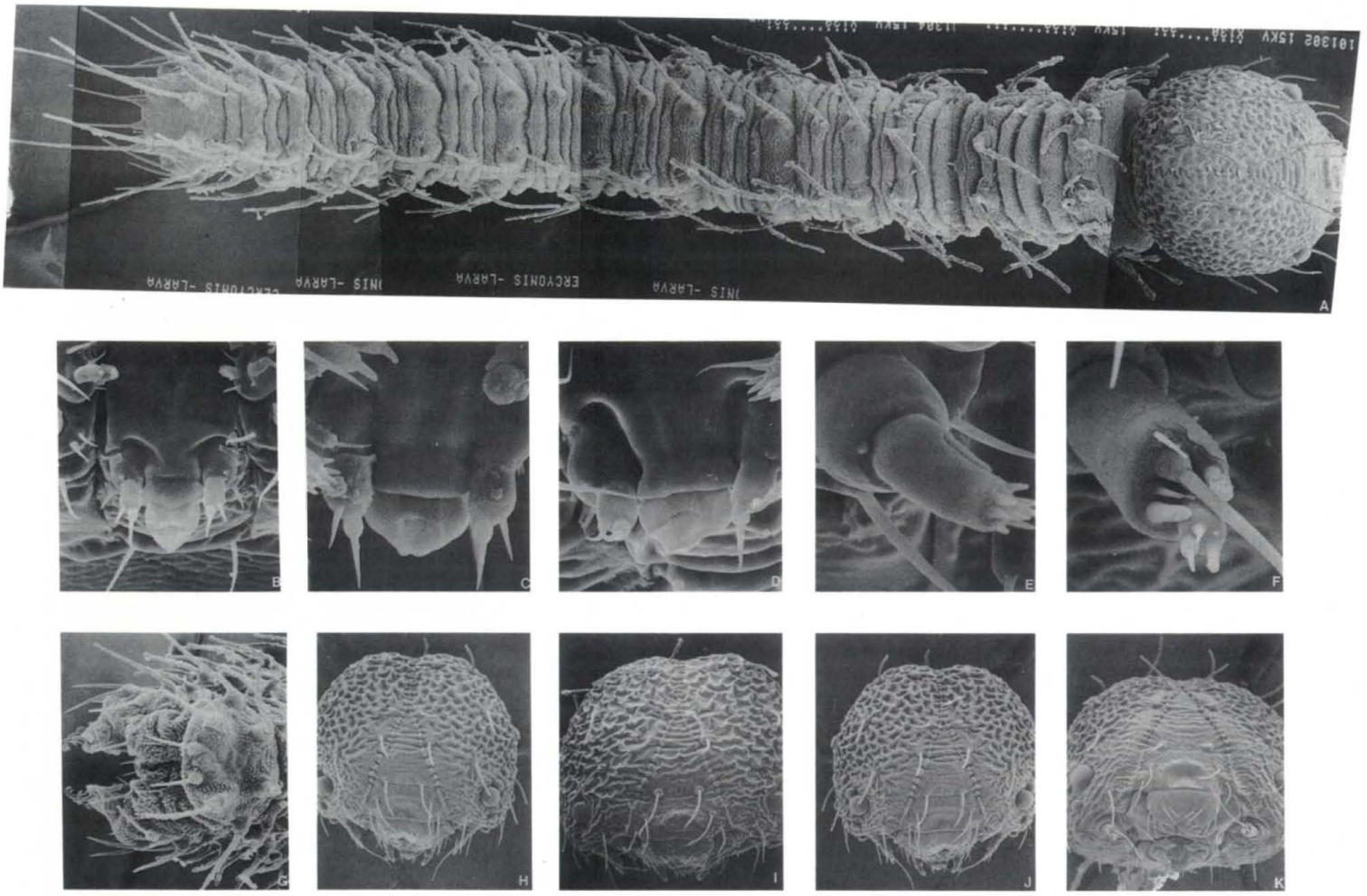


Fig. 9. Micrographs of first instar *Cercyonis* larvae: (A) *C. pegala* population from Ohio; (B) mouthparts of *C. pegala* from Colorado; (C) mouthparts of *C. pegala* from Ohio; (D) mouthparts of *C. oetus* from Colorado; (E) mouthparts of *C. pegala* from Colorado; (F) mouthparts of *C. pegala* from Ohio; (G) last segment of first instar larva of *C. pegala* from Ohio; H) headcapsule of the first instar larva of *C. oetus*; (I) headcapsule of the first instar larva of *C. pegala* from Ohio; (J) same as H; and (K) headcapsule of the first instar larva of *C. pegala*, Colorado.

Habitat and Flight Period

The habitat for *C. pegala* populations in Florida is primarily pine forest with an understory of oaks and different shrubs. Butterflies are never very abundant and do not spend very much time exposed in flight; they mainly secure themselves inside shrubs or on the bark of larger trees with their wings closed. They fly from early June until late September; thus many previous workers thought that *C. pegala* in Florida has two generations a year. However, it is not likely. From my observations, larvae, even those placed under the same conditions, grew very unevenly. The first male precedes the first female in hatching by approximately two weeks, and probably precedes by two months the hatching of the last female from a single brood of eggs. So, if a female lives 5-6 weeks in the wild as it lived in the laboratory, it is not surprising to find adult individuals of one generation flying both in June and September. The same expanded flight period for *C. pegala* populations is observed all over the United States; however, the flight period greatly depends on the average temperature of the locality, its elevation, and the quality of the particular year temperature-wise. Thus, one can find specimens in Texas as early as the beginning of May, while in more northern localities, females can be found in late October in certain years.

Fig. 10. The head and first thoracic segment of the mature larva of the *Cercyonis pegala* population from Gainesville, Alachua Co., Florida.

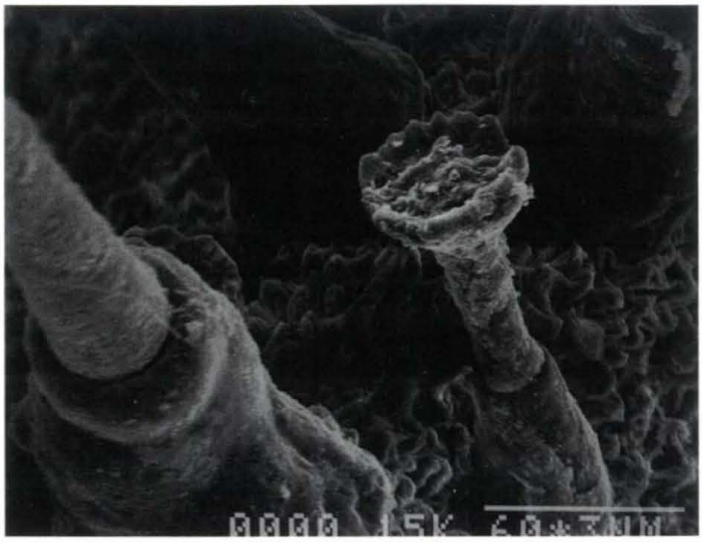


Fig. 11. Mushroom-shaped setae in the last instar of all of *Cercyonis pegala* maintained in culture.

In the summer of 1993, I had a chance to collect *C. pegala* in several different states. At Fruitland Mesa, Colorado, males were taken on 17 July, with only a few females being found. On 23 July, though, the percentage of females increased significantly, with males still dominant in numbers. In Rock Creek Canyon, Colorado, 7 males and 4 females were collected on 22 July. On 25 July in Siskiyou Co., California, at the elevation of 4,000 ft. (1700m), only fresh males were found, and three weeks later, mostly females were found on the Turnpike (I-80, exit 30) in Ohio. The females in Gainesville, Florida, were still fresh at the end of September.

In Colorado, *C. pegala* was found in an extremely dry environment of montane woodland scrub forest, adults hiding in the shade of juniper trees as at Fruitland Mesa on the western slope, or under oak trees as at Rock Creek Canyon on the eastern slope of the Rockies. They have a very fast flight when out in the open. Feeding and courtship mostly occurs during cooler morning hours.

In Ohio, populations of *C. pegala* adults are found in the open meadows along a highway (I-80). Meadows are generally described (e.g., Scudder, 1888; Klots, 1951; Howe, 1975; Pyle, 1981; Scott, 1986) as a habitat for the "*nephele*" (dark) form, while the "*alope*" (orange) form is mostly found in the woods. This Ohio population appeared to consist of individuals of both forms. The population was much more dense than ones I studied in Florida or Colorado. Butterflies had a slow flight and most were exposed to the open sky, either sitting on tops of the grass or making short flights of several feet.

Laboratory Observations

The average spotting patterns as well as wing sizes proved to be unique to every population sampled (Table 1 and 2).

As one can easily see even in the limited set of samples of mixed form "*alope*" / "*nephele*" populations, the ratio of different color forms in different *C. pegala* populations is different. The ratio in any particular *C. pegala* population has also been noted to not be consistant from year to year (Emmel, 1969). I examined major museum collections which contained short to long series of specimens from various *C. pegala* populations. Most of

Fig. 12. (A) First, (B) third, (C) first after hatching (under stereoscope), and (D) last instars, of *Cercyonis pegala* (Ohio, A-C; and Florida, D).

the populations in the Northeast, are represented by just one of the forms. The mixed populations of two color forms seemed to be found along the northeastern-central suture zone (Remington, 1968) from Nebraska to Pennsylvania, and then north along the East Coast to Maine. Some of the females in my experimental populations produced only "*nephele*" offspring, while some showed an introgression of the "*alope*" form, with domination of the "*nephele*" phenotype. With both forms being produced in a single brood under one set of environmental conditions, the difference between "*alope*" and "*nephele*" is very likely genetic and possibly due to a single gene with alternate alleles.

In the laboratory, freshly emerged adults, reared from eggs of wild-collected females, were tested in both screened cages and clear plastic boxes. Mating between the two basic color forms occurred as easily as mating between adults of the same color form, as it would be expected from polymorphic population found in the wild. A male would become ready to mate in three to four days after emergence from the pupa. Both males and females seemed to take active parts in courtship behavior, flapping their wings while facing in opposite directions. I suspect that both

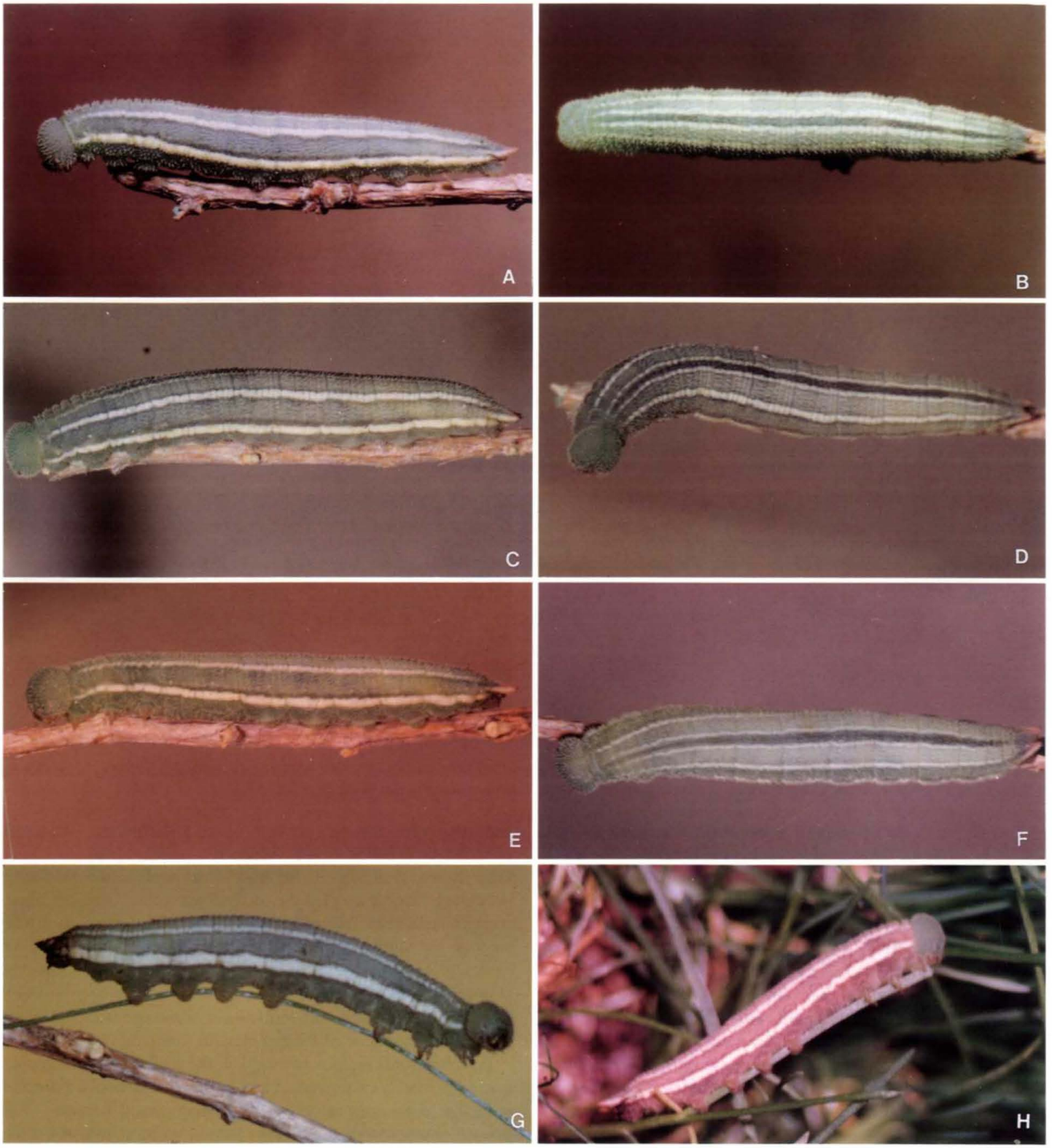


Fig. 13. Larvae of different species of *Cercyonis*: (A-B) *C. sthenele* (Boisduval); (C-D) *C. meadii* (W. H. Edwards); (E-F) *C. sthenele damei* (Barnes and Benjamin); (G) *C. sthenele paulus* (W. H. Edwards); (H) *C. sthenele masoni.* (Photographed by Thomas C. Emmel).

sexes release pheromones at this stage that are useful in courtship. Then the male places himself next to female, facing the same direction as she is, bends his abdomen 180 degrees, and mates. After copulation commences, he reverses his body, facing the opposite direction now from the female, and stays passive at all times during mating (Fig. 15B). Mating of individuals from Ohio always occurred within half an hour of 1000h, under a natural daylight cycle (the experiments were conducted in early May, when the time of sunrise six weeks before the summer solstice on June 22 is similar to that of the peak *C. pegala* flight period in early-mid August, following the summer solstice). Mating of individuals from Colorado always would occur within half an

Fig. 14. Variation in coloration of *Cercyonis pegala* pupae and larvae: (A-C) Pupae-offspring of a single female from a Colorado population of; (D-E) Mature larvae from Florida and Colorado, showing slightly different green coloration, determined by the time since last molting.

hour of 12 noon. In the case of both subspecies, mating would last for 1-1.5 hours. Observed in nature, the mating of the adults of the Colorado subspecies occurred at the same local time as at the laboratory in Florida (Table 3).

The intersubspecific matings occurred twice at 1000h and lasted for about an hour. However, both of these inter-subspecific-cross females laid infertile eggs, and each readily mated again with a male member of their own subspecies. That result probably indicates that no sperm was transferred at the first mating. Additional intersubspecific matings occurred around 1200h (noon), and eggs laid afterwards were fertile. While I lack sufficient matings to statistically verify it, it seemed that intersubspecific matings happened with less consistency than intrasubspecific matings. For example, a mating between *C. pegala* from Ohio and *C. pegala* from Florida never occurred despite a number of trials, and the adults failed to exhibit the start of courtship behavior. Of course, these two populations are phenotypically very different (males from the Florida population were even larger than females from the Ohio population, which never happens in natural populations of a *C. pegala* subspecies, where females are always significantly larger than males). In the case of Ohio and Colorado populations, at least three intersubspecific matings resulted in infertile eggs being laid, which never happened when mating occurred within a subspecies. Rejection of males by females in intrasubspecific pairings happened from time to time (see Fig. 16 for male scent scales used in mating behavior). The reason for this lack of acceptance was not clear. It might include the failure to provide particular mating conditions such as the amount of sunlight, proper food, or wrong age of participating butterflies. In nature, many males are probably eliminated from the reproduction process. These hybridization experiments resulted in individuals bearing wing patterns intermediate to those of the populations crossed.

It took several months to establish the technique for allowing mating to readily take place. I had to find the particular hour and particular conditions under which mating would happen. The procedure was to feed butterflies in the morning at around 0800h, then to keep them separated until 15 minutes before their supposed mating time (1000h or 1200h noon, depending on the subspecies). At that point, they were placed together in a one-pint cardboard container with netting on top, and the container was put against a window that opened to the outside sky. It is important that butterflies are not exposed to direct sunlight in the window setting: they can die within 5 minutes from overheating. In nature, they use the shade of the trees very effectively to regulate their body temperature.

TABLE 1. Spotting pattern and front wing size data for three different populations of *Cercyonis pegala boopis*.

Cercyonis pegala boopis, Frutland Mesa, Colorado population						
sex	LFW	LHW spotting	RFW	RHW spotting	Size of LFW	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	26.26	
male	3 3	1 3 1 1 3 2	3 3	3 3 2 3 3 3	26.17	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	28.43	
male	3 3	0 3 0 0 3 3	3 3	0 3 0 1 3 3	26.81	
male	3 3	0 1 0 3 3 0	3 3	1 1 2 3 3 0	26.81	
male	3 3	3 3 2 2 3 3	3 3	2 3 3 3 3 3	26.81	
male	3 3	3 3 3 0 3 3	3 3	1 3 3 0 3 3	26.52	
male	3 3	0 3 3 0 3 0	3 3	0 3 3 0 3 2	26.57	
male	3 3	3 3 3 0 3 3	3 3	3 3 3 2 3 3	26.57	
male	3 3	3 3 3 2 3 3	3 3	3 3 3 2 3 3	26.17	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	24.01	
male	3 3	3 3 2 2 3 3	3 3	3 3 3 2 3 3	26.58	
male	3 3	3 3 1 2 3 3	3 3	3 3 3 3 3 3	27.72	
male	3 3	3 3 1 2 3 3	3 3	3 3 3 2 3 3	26.15	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	25.09	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	27.04	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	26.79	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	26.98	
male	3 3	0 3 3 3 3 3	3 3	3 3 3 3 3 3	26.14	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	25.16	
male	3 3	0 0 0 0 3 3	3 3	3 3 3 3 3 3	26.27	
means	3 3	2.15 2.75 2.15 1.9 3 2.65	3 3	2.45 2.9 2.72 2.4 3 2.8	26.57	
					0.910724463	stdev
					0.829419048	var
female	3 3	0 0 0 0 3 3	3 3	0 0 0 0 3 3	27.69	
female	3 3	0 0 0 0 3 3	3 3	0 0 0 0 3 3	30.76	
female	3 3	0 2 2 0 2 2	3 3	0 2 2 0 2 2	30.76	
female	3 3	0 0 0 0 3 0	3 3	0 0 0 0 3 3	30.76	
female	3 3	0 0 0 0 3 3	3 3	0 0 2 0 3 3	29.68	
female	3 3	0 0 0 0 2 0	3 3	0 0 0 0 2 0		
female	3 3	0 0 0 0 3 3	3 3	0 0 0 0 3 3		
female	3 3	0 0 0 0 3 3	3 3	0 0 0 0 3 3		
female	3 3	0 0 0 0 3 0	3 3	0 0 0 0 3 0		
female	3 3	0 0 0 0 3 3	3 3	0 0 0 0 3 3		
female	3 3	0 0 0 0 3 0	3 3	0 0 0 0 3 0		
female	3 3	0 0 0 0 3 1	3 3	0 0 0 0 3 1		
means	3 3	0 0.18 0.18 0 2.8 1.64	3 3	0 0.18 0.36 0 2.8 1.9	27.69	
					1.336674979	stdev
					1.7867	var
Cercyonis pegala boopis population from Rock Creek Canyon, Colorado						
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	26.55	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	25.52	
male	3 3	3 3 3 1 3 3	3 3	3 3 3 1 3 3	23.71	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	25.9	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	25.68	
male	3 3	3 3 2 3 3 3	3 3	3 3 2 3 3 3	26.98	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	26.24	
means	3 3	3 3 2.85 2.25 2.71 3 3	3 3	3 3 2.85 2.71 3 3	25.9	
					1.050344615	stdev
					1.10322381	var
female	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	29.93	
female	3 3	3 3 3 0 3 3	3 3	3 3 2 0 3 3	29.93	
female	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	32.86	
female	3 3	0 0 0 0 3 3	3 3	0 0 0 0 3 3	32.47	
means	3 3	2.25 2.25 2.25 1.5 3 3	3 3	2.25 2.25 2 1.5 3 3	31.2	
					1.58705965	stdev
					2.518758333	var
Cercyonis pegala boopis form incana from Siskiyou County, California						
male	3 3	3 3 2 1 3 3	3 3	2 3 3 2 3 3	24 .39	
male	3 3	3 3 2 3 3 3	3 3	3 3 3 3 3 3	25.85	
male	3 3	1 3 0 0 3 3	3 3	1 3 3 0 3 3	24.17	
male	3 3	3 3 2 2 3 3	3 3	3 3 3 2 3 3	24.26	
male	3 3	3 3 2 1 3 3	3 3	3 3 2 3 3 3	24.04	
male	3 3	3 3 3 2 3 3	3 3	3 3 2 3 3 3	23.1	
male	3 3	3 3 3 3 3 3	3 3	3 3 3 3 3 3	24.23	
male	3 3	2 3 3 3 3 3	3 3	3 3 3 3 3 3	25.38	
male	3 3	3 3 1 2 3 3	3 3	3 2 0 3 3 3	24.14	
means	3 3	2.66 3 2 1.88 3 3	3 3	2.66 2.77 2.44 2.44 3 3	24.23	stdev
					0.721626786	var

TABLE 2. Form *alope / nephele* ratio in several populations of *Cercyonis pegala* in the Northeast (from museum collections) and in the offspring of laboratory-cultured broods from 4 females taken from an Ohio population.

Population	% of nephele	% of alope	% of intermed.	Total sample No.
VT, vic. Sandgate	13	70	17	24
NH, Grafton Co.	39	39	22	31
NH, Piermount	91	0	9	22
ME, Franklin Co.	86	0	14	14
ME, Naples	0	78	22	32
ME, Gilecard	39	35	26	23
NY, Bedford	17	83	0	7
NJ, Sussex Co.	0	0	100	20
Total in Ohio pop.	65	15	20	60
female one: nephele	50	25	25	8
female two: nephele	100	0	0	4
female three: neph./intermed.	37	24	37	27
female four: neph./intermed.	100	0	0	15

TABLE 3. Mating time of *Cercyonis pegala* in the laboratory cultures.

The butterfly #	population	Mating period		
771-11-5 fem. x 770-1-6 male	Ohio xOhio	11:00-1:00	fertile	
771-11-6 fem. x 771-11-5 male	Ohio xOhio	11:00-1:00	fertile	
770-2-2 fem. x 770-2-5 male	Ohio xOhio	10:10-11:30	fertile	
771-11-3 fem. x 770-2-8 male	Ohio xOhio	11:00-12:00	fertile	
771-11 fem. x 771-11-2 male	Ohio xOhio	10:30-12:00	fertile	
771-11-10 fem. x 770-1 male	Ohio xOhio	10:10-12:00	fertile	
771-11-15 fem. x 771-11-9 male	Ohio xOhio	9:50-11:30	fertile	
771-11-16 fem. x 771-12 male	Ohio xOhio	10:00-12:30	fertile	
770-2-8 fem. x 770-2-12 male	Ohio xOhio	11:00-1:00	fertile	
770-2 fem. x 770-2-6 male	Ohio xOhio	10:00-11:30	fertile	
770-2-3 fem. x 770-1-4 male	Ohio xOhio	10:45-12:25	fertile	
771-11-2 fem. x 713-11-4 male	Ohio x Colorado	12:00-1:30	fertile	
770-2-5 fem. x 713-11-4 male	Ohio x Colorado	11:00-12:30	fertile	
770-2-7 fem. x 713-11-4 male	Ohio x Colorado	12:35-2:00	fertile	
771-11 fem. x 713-11-2 male	Ohio x Colorado	10:20-11:00	infertile	
713-1 fem. x 771-11 male	Ohio x Colorado	10:20-11:00	infertile	
All the intrapopulational crossings of Colorado population, total of five, started between 12:00 and 1:00.				

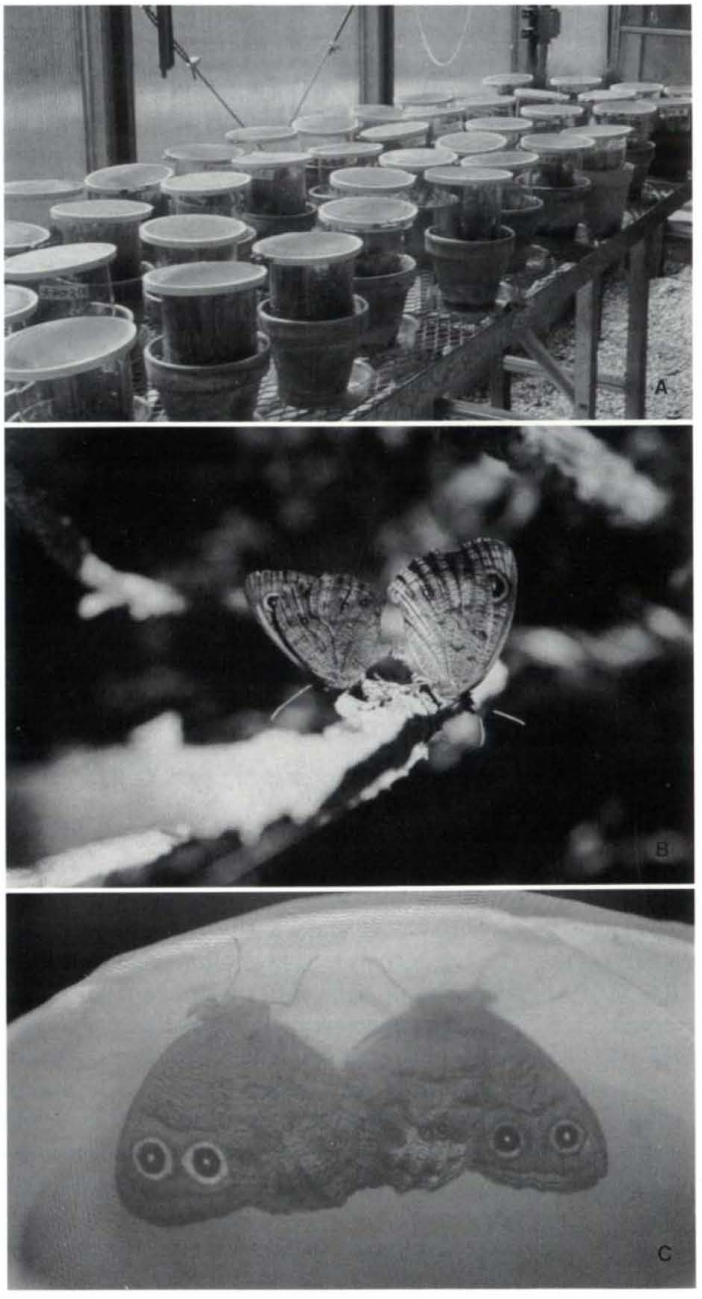


Fig. 15. (A) The rearing setup used in the greenhouse culture at the University of Florida: the pot with freshly-grown Kentucky bluegrass is covered with a glass cylinder with netting on top. First instar larvae are released into the pot and, and are allowed to mature on the grown grass. (B) *Cercyonis pegala boopis* (Colorado) male and female copulating in nature. (C) Hybridization of Colorado male with Ohio female in a small ice cream container in the laboratory.

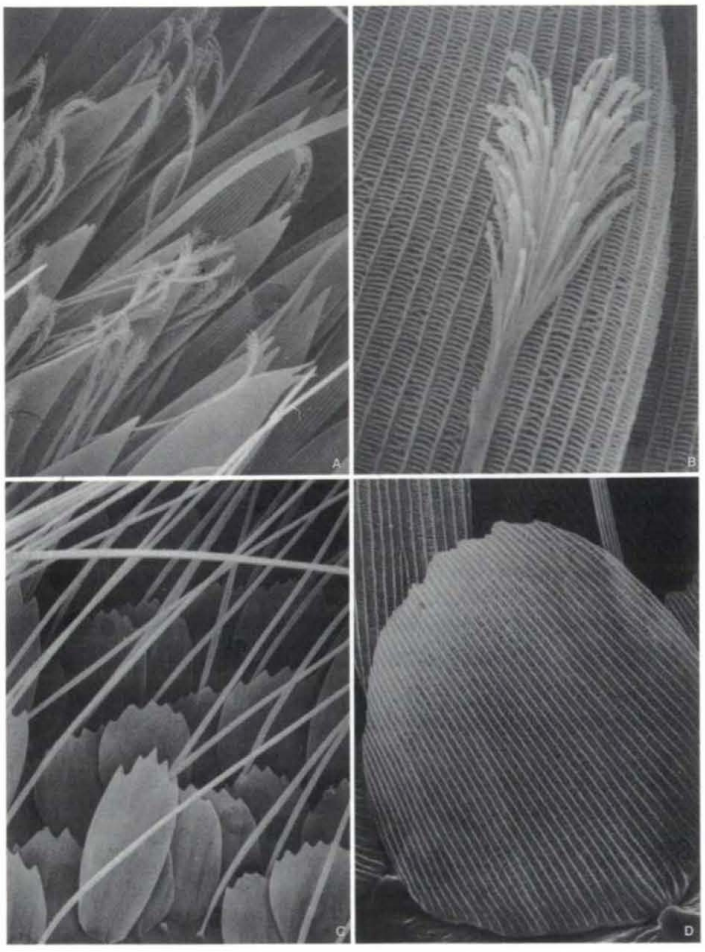


Fig. 16. Microphotographs of the androconial scales found in males of different *Cercyonis* species; pheromone release from the wing surface seemes to be an important part of successful copulation, however, the morphology of those scales, sometimes used as characters by systematists, proved to be similar in all the *Cercyonis* taxa studied on both specific and subspecific level.

Three rearing cycles were conducted under these regimes. The first one took place in November 1993 inside the laboratory building under a 12-hour daylight cycle. The pots with Kentucky bluegrass were placed under banks of 40-watt Gro-Lux R fluorescent lights. The average temperature in the room was around 21°C. It took larvae two months to mature under these conditions. Even with an abundance of food, adult butterflies in the first rearing cycle came out much smaller than their natural ancestors (Fig. 17). The second rearing cycle was conducted in a 10 x 20 foot greenhouse in April 1994, at the average diurnal temperature of 35°C. Larvae matured much faster (in 4-6 weeks) under these conditions. The resulting butterflies came out equal to and sometimes larger than their natural ancestors. In nature, we find the equivalent size different in geographically separated populations. Thus, in California, *C. p. incana* (W. H. Edwards) shows a much smaller average size than *C. p. boopis* (Behr). This small size is probably a result of lower temperatures and shorter annual growing periods at higher-elevation sites. Judging from the size of head capsules of skin casts left after pupation of the first laboratory brood, I found that *C. pegala* was capable of skipping the last (sixth) instar under the influence of extreme conditions during rearing, such as unusually cold temperatures, insufficient light, or poor quality of food (the grass was too old

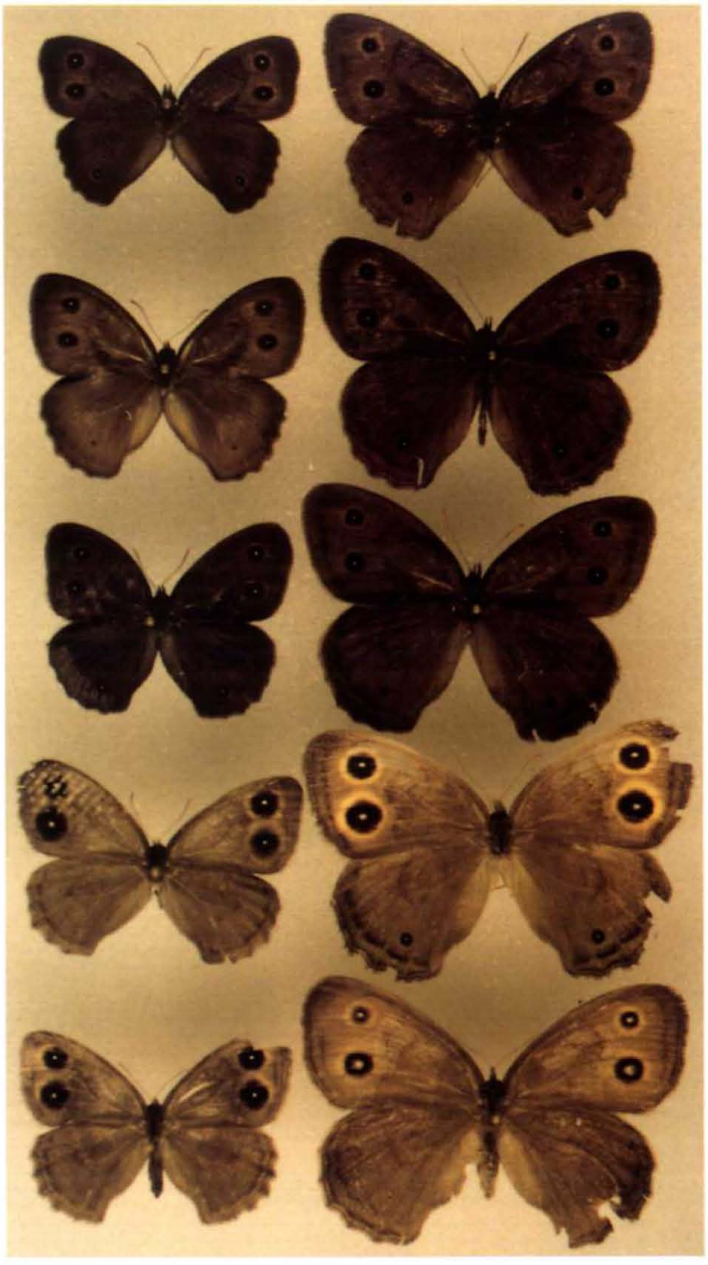


Fig. 17. Comparison of normal-sized adults and unusually small *Cercyonis pegala* population reared under severe laboratory conditions of low temperature and poor food quality. The butterflies on the left probably underwent only five larval instars in the laboratory, while their natural-sized ancestors (on the right) underwent six larval instars.

at the end of rearing and almost completely eaten, leaving larvae to feed on malnutritious lower parts of leaves).

Another observation of biological difference is worth mentioning. The first instar larvae of the Colorado *C. pegala* population would start eating right away after they were transferred to 20°C from the refrigerator (5°C). Larvae of the Ohio population would not start feeding until the second or third week after transferral. Accordingly, their development period through the six larval instars was delayed and they would hatch from the pupa two weeks later than their Colorado relatives. Larvae of the Colorado population also proved to be much more durable than those from Ohio; they survive freezing and desiccation much better than their Ohio relatives.

The third rearing was attempted in early September 1994 under the same greenhouse conditions as the second rearing cycle. However, eventhough the temperature was high, and there was plenty of light, larvae would not start feeding, sitting passively on the grass. Only three larvae from the Colorado population and one hybrid larva began feeding. It took me a month to realize, that, despite the overall favorable conditions, the day time at this time of the year is getting shorter, and that probably triggered the diapause state. I transferred some of the pots with larvae into the cooler laboratory conditions (25°C) but with 12-hour light cycle. The larvae in these pots started feeding within a week, while those in the greenhouse (35°C) stayed in diapause. This result puts in doubt the concept of diapause being triggered entirely by temperature. Even the absolute day-length seems not to matter. What triggers diapause, or, as in our case, triggers coming out of diapause, is the minute change in the day-length. If the larva encounters an increase in daily day-length, it probably means the advent of spring in the annual cycle and it is relatively safe to come out and start feeding. If the larva encounters a decreasing daily day-length, it would anticipate a summer-fall transition and the need to go into diapause for the winter. One can see how the diurnal periodism, as a trigger, could be selected for Colorado populations, where temperature is subject to wide fluctuations and it might even freeze on occasion during the summer. It would require some additional study to determine whether the change in day-length alone or in combination with other factors plays the key role in breaking the diapause.

MOLECULAR BIOLOGY

1. ANALYSIS OF CUTICULAR HYDROCARBONS

Cuticular hydrocarbons (HC) serve many different functions in insects. They comprise a significant portion of the cuticular lipids that prevent desiccation. They are also important in chemical communication, serving as sex attractants and aphrodisiacs, as species and caste recognition cues, and as territory-marking and alarm pheromones (Howard, 1982). Thus, it was determined that C 23 olefin in the cuticular HC complex of the house fly is a close-range sexual stimulant responsible for initiating the mating strike of the male. Cuticular HC on termites serve as cues for caste and species recognition (Howard, 1987). HC released by mosquito larvae serve as overcrowding pheromones: they are toxic for conspecific first-instar larvae. Parasitoids use HC to mark already-parasitized hosts.

HC are synthesized in cells associated with the epidermal layer and are a significant part of the wax layer of the cuticle. It is hypothesized that hydrocarbons may reliably identify individuals of otherwise morphologically similar species. Analysis by gas chromatography (GC) gave definitive results for identification of *Blattella* cockroach species (Orthoptera) of North America, tsetse flies (Diptera) and honeybees (Hymenoptera) (Carlson, 1988). Carlson and Yocom (1986) presented evidence for species specificity of cuticular HC in tephritid fruit flies. Different species of mole crickets also showed distinctive HC patterns (Castner and Nation, 1986).

An amount of cuticular HC sufficient for GC analysis can be obtained from as little material as one Varroa mite (Acari) (Nation *et al.*, 1992) by simply rinsing live, frozen or even dead and dried specimens from the collection with an organic solvent like hexane or pentane (Carlson, 1988). The extract is then run on a GC and the results of mass spectrographic analysis can be compared with the library of known chemicals stored in the computer. That makes it extremely convenient for separation of cryptic species: one can get an answer in less than an hour.

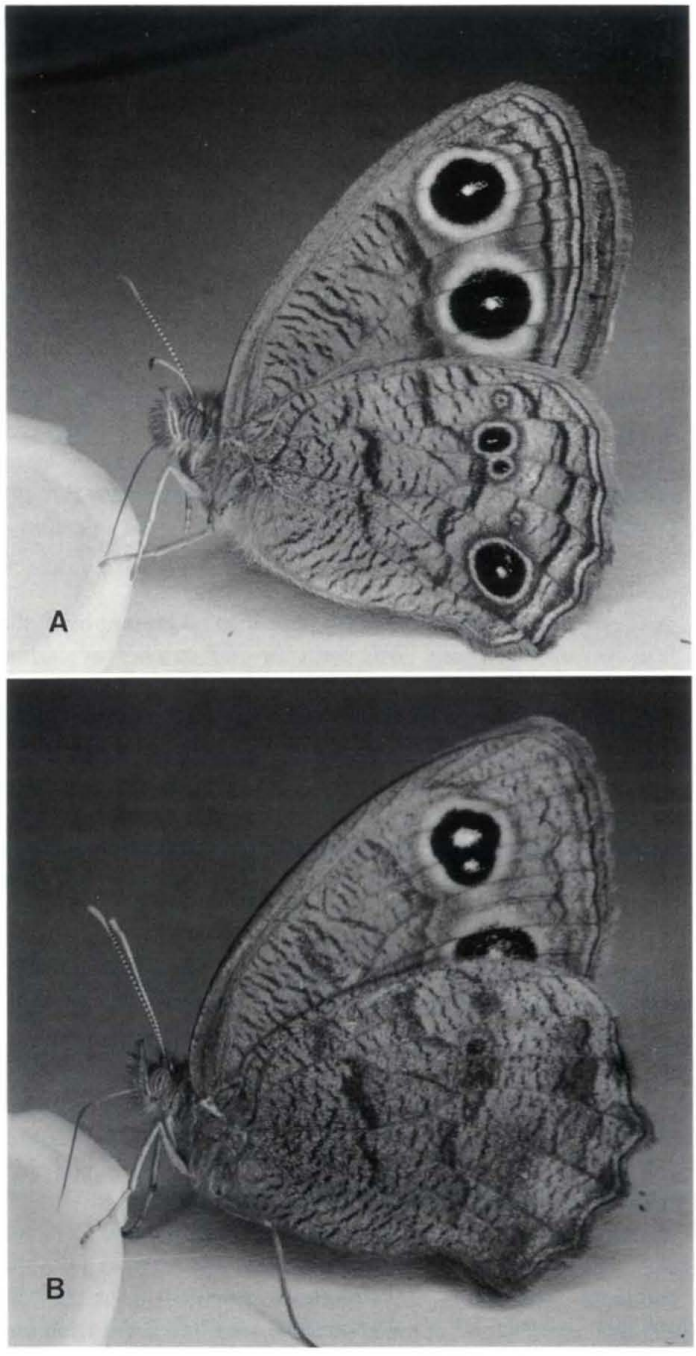


Fig. 18. (A) *Cercyonis pegala* population from Fruitland Mesa, Colorado, has males with a very distinct phenotype, because of the unusually large size of the fifth ocellus ventrally. (B) Rock Creek Canyon, Colorado, population with female easily distinguished by the absence of ocelli, yet in current nomenclature, belongs to the same subspecies as the male above. This female also shows the pupil doubling of the first ocellus on the dorsal front wing surface, one of the characters used to define the subspecies called "*wheeleri*."

As a part of my attempt to clarify the systematic picture of this group, I tried to analyze cuticular HC from several subspecies of *C. pegala*: *C. p. boopis* from Colorado, *C. p. abbotti* from Florida, and *C. p. olympus* from Ohio. I also analyzed HC of *C. oetus* from Colorado as a close relative of *C. pegala*.

Materials and Methods

Abdomens from live adults were cut off and frozen in a liquid nitrogen tank, except for specimens from Florida which were analyzed fresh. They were rinsed in a sufficient amount of pentane to extract the sample. The samples were filtered through a short column of silicit acid, which eliminated all oxygen-containing molecules (such as fatty acids, alcohols, sterols, acetylglycerols) from the sample. Samples then were evaporated to 0.5 ml. Commercially available standards and experimental samples were analyzed on a 25m x 0.25mm fused silica capillary column with bonded polydimethylsiloxane coating in a Shimatzu G14-A gas chromatograph with capillary injector port and flame ionization detector (FID). Initial temperature of the column was 200°C and was programmed to rise to 300°C at a rate of 4°C/min. It was to hold at 300°C for the duration of the run. The injection was splitless. The linear flow rate of helium carrier gas through the column was 30cm/sec and followed 30 sec. after injection.

Results

All typical insect HC (C22-C30) appear to be present in all the subspecies of *C. pegala*. HC with an odd number of carbon atoms (C) are usually in slightly different quantities than ones with an even number of carbon. Local populations of *C. pegala* showed some variation in the relative quantity of different HC and significant variation in the total quantity of HC per specimen. That finding probably has to do with the age of the butterfly, the conclusion which is also confirmed by the failure to extract HC from the older museum specimens; only the most recently collected adults showed some trace of HC. There seems to be as much variation between the specimens of the same subspecies as between specimens belonging to different subspecies. Thus, I found no distinct HC pattern which could characterize each of the subspecies. In both specimens of *C. oetus* analyzed, there was no C23 present, which, however, was abundant in *C. pegala* (amount of this HC also seemed to be the most variable of all the HC among different subspecies of the *C. pegala* complex). Whether the amount of C23 is at a characteristic level in particular taxa should be confirmed by repetitive analysis.

The compounds whose peaks arose between C24 and C25, and which were separated and analyzed from the sample as polar compounds, appeared to be contaminants coming off the glassine envelopes, in which all of the specimens analyzed spent from one to several hours. The shape of their peaks on the GC resemble those of the HC, but the closest mass spectrum found in the library was of 8-nonenoic acid, 9-(1,3-nonadienyloxy)-, methyl ester. Besides these compounds, the washing from a glassine envelope contained some of the C24-C30 hydrocarbons.

The separate washings from the wings, thorax, and abdomen of the same specimen of the *C. p. pegala* from Florida showed that wings and thorax (most probably wings) contain some heavy HC with C35 and C37, which are not found on any of the abdomens.

Discussion

Cuticular HC serve as a good character for separation of the sibling species in some insect groups, such as mole crickets (Orthoptera) or honeybees (Hymenoptera). However, for the group of butterflies studied, they appeared to be useless as a systematic tool. This finding should not discourage anybody from application of that technique to other groups of insects, but it proves that HC pattern should be considered as just one of many available characters to study, which can be as variable as any of the more commonly used morphological characters.

The example with glassine envelope contaminants shows how careful one should be collecting and storing material for GC study. Even a few minutes of contact with a chemically rich surface such as wax paper or a plastic container can introduce strong contamination into the sample. The best material for the storage containers seems to be carefully pre-cleaned glass.
There are examples from previous studies where insect specimens from old museum collections were successfully analyzed. However, the present study shows that the best material for cuticular hydrocarbon analysis is either fresh or frozen specimens, and that some hydrocarbons and sometimes all the hydrocarbons can be lost with the passage of time.

2. ALLOZYME ELECTROPHORESIS

Allozyme electrophoresis is a powerful technique for establishing the relatedness of individuals or populations, finding sibling species, and creating phylogenetic trees. In cases where the question arises as to whether allopatric populations belong to the same or different species, electrophoresis might be also quite useful. Unlike traditional analysis of genetic relatedness based on allele frequencies, the analysis of the fixed allelic differences would be used for detecting allopatric species (Richardson *et al.*, 1986).

Among vertebrates, populations of the same species rarely differ at more than 14% of loci (Richardson, 1986). Therefore, if allopatric populations differ at more than 20% of loci, they could, with a high degree of confidence, be considered separate species. The converse is not true, because many species differ at less than 14%.

Materials and Methods

It is only necessary to screen a few individuals (three to five per population) for studies of specific or subspecific allozyme differences. This is because for an enzyme locus, each diploid individual carries two copies of each gene, and for each locus heterozygotes can be distinguished. Thus we have two or more independent (multiple alleles) measurements of each character for each individual. Also, electrophoretic studies have shown that most populations are monomorphic at an average of 85% of isozyme loci: a single individual is representative of the whole population for 85% of characters. Finally, even for the 15% of loci that are polymorphic, a single individual will be partly representative of the whole population. For a locus with two alleles at frequencies of 0.8 and 0.2, there is a 96% chance that a single individual will carry at least one copy of the more common allele (Richardson *et al.*, 1986). So I used four individuals from Ohio; two from Fruitland Mesa and one from Rock Creek Canyon, Colorado; two from Gainesville, Florida, and two from Idaho.

The equipment and materials used were kindly supplied by Thomas C. Emmel at the University of Florida. The set of sixteen enzymes used by him for his studies in population genetics of *Cercyonis oetus* was analyzed by me for studies on *C. pegala* samples, and included the enzymes listed in Table 4.

The materials used were abdomens of adult butterflies frozen in the liquid nitrogen in the field and stored at -70°C in an ultrafreezer.

TABLE 4. Enzymes used for electrophoresis of *Cercyonis pegala* population and number of loci corresponding to those.

Enzyme	No. of loci	Enzyme	No. of loci
ACON	2	HK	2-3
AK	2	IDH	2
GAPD	1	LDH	3
GOT	2	MDH	2
GPD	1-2	ME	2
G6PD	1	MPI	1
GPI	1	6PGD	1-2
HBDH	1	PGM	3-4

TABLE 5. Scoring of electrophoretic gels of *Cercyonis pegala*: A-C represent different alleles; AA or BB represent homozygous individuals; AB or BC represent heterozygous individuals.

ENZYME	ACON	G6PD	HBDH	LDH	MDH	ME	MPI	IDH	GPD	HK	6PGD	PGM	GAPD	GOT	GPI
INDIVID.															
OHIO 1	AB	AA	BB	CC	CC	AA	AB	AA BB	AA	AA -	BB	AC	AA	BB BB	BC
OHIO 2	BB	AA	BB	AA	CC	AA	BB	AA AA	AA	AA -	BB	BB	AA	BB BB	BC
OHIO 3	BB	AA	BB	CC	CC	AA	BB	AA BB	AA	AA -	BB	BB	AA	BB BB	BB
OHIO 4	AB	AA	BB	AA	BC	AA	AB	AA AB	AA	AA BB	BB	BB	AA	BB AB	BB
COLO 1	BB	AA	BB	AA	BC	AA	AB	AA AA	AA	AA -	BB	BD	AA	AA BB	BC
COLO 2	BB	AA	BB	BB	BC	AA	BB	AA BB	AA	AA AA	BB	AA	AA	CC BB	BB
FLORIDA 1	BB	AA	AA	AA	BB	AA	BB	AA AA	AA	AA BB	BB	BB	AA	CC BB	BB
FLORIDA 2	BB	AA	BB	AA	CD	AA	BB	AA AA	AA	AA -	-	BB	AA	BB BB	AB
IDAHO 1	BB	AA	BC	AA	AA	AA	BB	AA AA	AA	AA AA	AC	BB	AA	CC BB	BB
IDAHO 2	BB	BB	BB	AA	BB	AA	AB	AA BB	AA	AA -	BB	BB	AA	BB BB	BB
COLO*	BB	AA	AA	BB	BB	AA	BB	AA BB	AA	AA BB	AA	BC	AA	BB BB	BC

Results

The results of these experiments are summarized in Table 5. As can be seen there, every locus was represented by at least two alleles in the population, and none was fixed (monomorphic). Prior to beginning this electrophoresis analysis, I accepted a null hypothesis that the populations of *C. pegala* are of the same species. In this case we would find the fixed allelic differences in more then 20% of the loci, the null hypothesis would be rejected. I have not found fixed differences in any of the loci. That does not mean that those populations belong to the same species, however, no evidence has been acquired which would allow me to reject the null hypothesis. Table 5 shows some examples of actual electrophoretic gels obtained during analysis.

CONCLUSIONS

Many of the points made in this study of *Cercyonis pegala* are very arbitrary, as well as speculative. However, this study, leaving many questions open, shows the possibility of having alternative points of view on many established approaches in systematics.

The most important findings and conclusions of this study are:

1. The subspecific names of *C. pegala* across the eastern United States are synonymized under one nominotypical name of *Cercyonis pegala pegala* (Fabricius) with the recognition of the existance of two major wing-pattern forms as "*pegala*" and "*nephele*."
2. In my review of the historic usage of the subspecies concept by butterfly systematists working on *C. pegala*, I conclude that the present widely used practice of naming subspecies on the basis of one or two variable adult characters in disjunct populations is leading to chaos and to an unusable taxonomic system. I propose that a phylogenetic approach represents a more satisfactory and uniform method of delineating subspecies. The latter approach would eliminate much of the subjectivity presently involved in naming the subspecies. Patterns of geographic variability involving alternate states of one or several minor characters may be best referred to as clines, polymorphisms, or other evolutionary phenomena, rather than named subspecies.
3. The increase or decrease of the length of the light period of the day is recognized as the diapause-breaking or diapause-initiating mechanism in *C. pegala* larvae. That contradicts the previously existing opinion that the temperature plays a major role in those processes.
4. Certain speculations were made on possible evolution of the genus *Cercyonis*. It is concluded that *Maniola* and *Hyponephele* are the closest presently-existing relatives of the genus *Cercyonis*. It is also proposed that the speciation of *Cercyonis* took place at the time of the last Pleistocene glaciation, in a series of geographic movements involving local extinctions and repopulating of the North American continent. It is also proposed that *C. pegala* gave rise to the rest of the *Cercyonis* species and that the loss of one larval instar may have played some role in this process.
5. Cuticular hydrocarbon analysis and allozyme electrophoresis were shown to be of no use in differentiating species and subspecies of *Cercyonis*.

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