INVESTIGATIONS ON RACIAL BIOLOGY ESPECIALLY IN LEPIDOPTERA

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ABSTRACT OF THE PRIMARY RESULTS

Genetic and wild population studies have shown that the two races of Colias chrysotheme are different on a multiple-factor basis, these including physiological differences as well as a color difference. Sterility in the crosses may be largely related to diet differences between the races though evidence is presented to show that the hybrid segregants are less viable than the parental types. A wild population where intercrossing occurs shows about 10% of intermediates - mostly fertile.

A dominant autosomal gene for white female color is found in wild populations of both races. Genetic results indicate that it is probably homologous in the two races and is interchangeable between them. The gene may be either lethal or semi-lethal with certain modifiers when homozygous dominant. Within each race the gene is most abundant in the northern populations as compared with the southern.

The history of the recent establishment of the orange race in the area east of the Mississippi River is reviewed; the evidence shows that the planting of alfalfa in that area has been the primary factor which has encouraged the extension of range of the butterfly.

The causes of genetic alterations in wild popualtions has been studied by means of the white females. Genetic population changes are described which have been caused by migration, environmental selection, random fluctuations and differential development rate of the genotypes.

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The Nomenclature of the *Colias* *chrysotheme* Complex in North America (Lepidoptera-Pieridae)

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Genetic and population studies on the *Colias* *chrysotheme* complex of North America indicate that the group is divisible into two natural subgroups or races. One race is visibly differentiated from the other most easily by the presence of orange pigment on the upper surface of the fore wing; the other completely lacks the pigment in this location. Correlated with this character are several physiological differences relating to growth and diet; these phenomena will be considered in detail in other publications (see abstract, Hovanitz, 1942).

Owing to considerable interbreeding, these two major groups exchange sufficient gene materials in some populations to be identical in morphological characters. Therefore, it is doubtful that the two groups should be designated as taxonomic species, but instead will be designated "orange-race" and "yellow-race" respectively.

The name *chrysotheme* refers to a Palearctic species of Colias which is apparently identical in morphological characters with the North American forms. It may be found later with additional information that their classification as a unit should be reconsidered. The name has been revived for use for the North American forms by Clark (1941); it has been in use by many of the early nineteenth century entomologists and by Godman and Salvin (1889) for the orange-race. The author of *keewaydin*, W. H. Edwards, stated that "*keewaydin* = *chrysotheme* except that *chrysotheme* has not the extreme variability of *keewaydin*". *Keewaydin* is a seasonal variation of the orange-race. With the information available at present, *chrysotheme* seems to be a reasonable name for the yellow and orange races of North America.

The yellow-race can be subdivided into five entities corresponding to five geographic zones. These blend one into the other so that specimens from intermediate locations can be designated by the name applying to one or the other adjacent geographical zone. The variation in the butterflies within a zone is tremendous, due to seasonal climatic effects on the phenotype of the adult butterfly as well as to genetic variations within the populations. Some populations are now in the process of genetic alteration owing to recent and present exchange of genes with the orange race. It is doubtful that a new name should be applied to these new products of genetic segregation. Instead, it is better to recognize the change which is taking place and to withhold naming the new race until such time as the alteration is apparently completed.

Variations in the orange race are plentiful but do not seem to have geographical significance ~~in~~ on a genetic basis. The large, heavily orange-pigmented material from the Mississippi Valley may be genetic on an adaptational basis; but, as these forms occur wherever the humidity and temperature conditions are high enough, no convenient end is achieved in applying a Latin name to them.

The use of the binomial or trinomial name in this group must remain a matter of convenience depending upon the use to which the name is put. The orange-race might more practically be designated *Colias eurytheme* by the economic entomologist when studying the destructive nature of this form on alfalfa. Since none of the five geographical segregations of the yellow race ~~is~~ are of economic importance, a general name to cover them all is not then immediately necessary. The usual taxonomic method would be to take the oldest name *philodice* and to subordinate the others under it. The following system has been adopted as being most practical for general use and for showing genetic relationships:

Colias chrysotheme Esper

NORTH AMERICAN RACES

YELLOW-RACE

Colias chrysotheme philodice Godart, Enc. Meth. 9:100, 1819.*

Synonyms: notatus Meg., europome Haw., nig Str., nigridice Scud., melanic Skin., nigrina Stkr., miscidice Scud., nigrofasciata Reiff., hybrida Stkr., luteitincta Wolc., virida Stkr., inversata Nak., rothkei Reiff., raritus Gund., serrata Chermock, plicadata Nak., albida Cherm., alba Cherm., minor Cherm., ehrmanni Cherm., alba Streck., pallidice Scud., albinic Skin., suffusa Ckll., nigrofasciata Reiff., anthyale Hbn.

geographical distribution: Georgia to Labrador west through the eastern portion of the Mississippi Valley and north to Hudson Bay.

Type locality: Virginia*

Colias chrysotheme hageni Edwards, Papilio 3:163. 1883.

Synonyms: nigricosta Cherm., laurae Cherm., autumnalis Ckll., eriphyle (various).

geographical distribution: Texas, New Mexico, Utah and eastern California north to Montana, Idaho and Washington.

Type locality: "Southern Colorado to Montana and Dakotah", restricted by Barnes and McDunnough to Pueblo, Colo. No one type specimen.

Colias chrysotheme eriphyle Edwards, Trans. Amer. Ent. Soc. 5:202. 1876.

Synonym: kootenai Cockle.

geographical distribution: Northern Washington and Idaho, Rocky Mtns. of Alberta, southern and central British Columbia.

Type locality: Lake Lahache, British Columbia.

Colias chrysotheme vitabunda Hovanitz

Synonym: kootenai (various but not Cockle).

geographical distribution: Alaska, Yukon Territory, Northwest Territories, northern British Columbia.

Type locality: "Mt. McKinley National Park, Alaska".

Colias chrysotheme guatemalena Rober, in Seitz, Grosschmetterling der Erde. 5:91. 1907.

Synonym: philodice (Godman and Salvin)

geographical distribution: high elevations in Guatemala.

Type locality: no types, locality clearly Guatemala.

ORANGE-RACE

Colias chrysotheme eurytheme Bdv., Ann. Soc. Ent. Fr. 2:286. 1852.

synonyms: ariadne Edw., keewaydin Edw., intermedia Ckll., alba Stkr., fumosa Stkr., rudkini Gund., amphidusa Bdv., californiana Men., flava Stkr., unicitrina Gund., pallida Ckll.

geographical distribution: southern Mexico to southern British Columbia and Hudson Bay from the Atlantic to the Pacific oceans.

Type locality: California

Names applying to individual aberrants, to white forms, to melanic forms, to seasonal forms, and so forth have been placed in the synonymy under the subspecific name applying to the geographical region from where the material came. This does not preclude their use by persons who find it convenient to use such names, provided the latter are available under the rules of nomenclature. The author finds no convenience in their use but rather a great inconvenience. Variations of the orange-race are placed under that race; likewise, intermediates between the orange- and yellow-races are placed in that place.

The differences between the above races or subspecies with respect to combinations of genetic characters possessed by each will be covered thoroughly in another publication. The present revision of the nomenclature is essential before the population work can be adequately described.

Description of the New Subspecies

Colias chrysotheme vitabunda Hovanitz, ssp. nov.

This is the northern representative of the yellow-race of *chrysotheme* in North America. As such, it is characterized by its slightly smaller size, by its relatively narrow melanic border, by its rounded wings, by a heavy deposition of red pigment on the extremities, by a relatively light-colored hind-wing cell spot, by a melanic suffusion on the under-surface hind wing, by a reduction in size or complete disappearance of the submarginal row of spots on the under-side hind-wing, by a very high frequency of white females in the populations (95% in Alaska, 71% in Yukon Territory, Northwest Territories and N. W. British Columbia, N =77), by a nearly complete obsolescence of the inner portion of the melanic border on the upper surface of the female and the entire border on the hind wings and by a high frequency of white females with a white rather than orange or yellow hind-wing cell-spot. The males have a rather heavy deposition of orange pigment on the under-side hind-wings and apices of the fore-wings. There is a higher degree of intermediacy between the yellow and white females in this race than in other races.

The holotype female selected is white, as this genetic mutant is more abundant than the yellow. The variation in color of the hind wing cell spot (upper side) in the white females is from bright orange to pale yellowish-white. The holotype is of nearly the whitest type. By analogy with the genetic results in the orange-race, this possibly means that the individual was homozygous for the dominant gene controlling the white character. The pterine pigmentation of the underside hind wings and apex fore wings is very light orange-yellow. It will be shown later that these two characters are genetically correlated.

The allotype male differs from eriphyle mainly in its smaller size and the more complete reduction of the submarginal row of spots on the under side of the wings. From hageni, in addition to the above, it differs in a heavier orange suffusion on the under side of the wings, in its rounder wings and narrower marginal melanic band.

Holotype female and allotype male: McKinley National Park, Alaska, July 18 to August 9, 1930. Frank Morand collector. J. D. Gunder Collection, Accession no. 34998, American Museum of Natural History.

Paratypes: 12 ♂♂ same data as above; 11 ♂♂ same locality but July 29, 1931 and "collector unknown"; 4 ♂♂ July 15-20, 1931, "collector unknown" in Los Angeles Museum. 10 white ♀♀ same data as Holotype; 4 white ♀♀ July 20-30, 1931, "collector unknown" in Los Angeles Museum; 1 white ♀ August 9, 1930 in Los Angeles Museum, "collector unknown".

That portion of the following material which has been examined is indistinguishable from the above type material, but in order to avoid confusion in type locality, has not been designated as part of the paratype series.

Alaska: "Alaska" - 5 ♂♂; Mt. Dewey, 5000', 3 ♂♂; Eagle, Rampart, Kuskokwim River, Circle, Ft. Yukon, 61 ♂♂, 1 yellow ♀, 10 white ♀♀; Circle, 5 ♂♂; Eagle, 14 ♂♂, 6 white ♀♀; Chitine, 1 white ♀; Ft. Yukon, 1 white ♀; Skagway, 10 ♂♂, 5 white ♀♀; Alfred Creek Camp, 1 white ♀, Mt. McKinley National Park 10 white, 3 intermediate and, 2 yellow ♀♀.

Yukon Territory: Whitehorse 52 ♂♂, 3 white ♀♀, 3 yellow ♀♀; Dawson, 33 ♂♂, 3 white ♀♀, 1 yellow ♀; Campbell Creek, Pelly River, 1 ♂; Pelly River near Hoole River, 1 ♂; Klotassin River 1 ♂, 1 yellow ♀.

Northwest Territories: Great Slave Lake, 3 white ♀♀.

British Columbia: "Northwest B. C.", 2 ♂♂; Atlin, 1 yellow ♀, 1 white ♀; Monarch mountain, 1 specimen; Pike River, Atlin, 5 white ♀♀.

Discussion of the Names Kootenai Cockle and Alberta Bowman

The name *kootenai* was applied, without much description, to *Colias* occurring in the vicinity of Kaslo, B. C. early and late in the season as compared with *eriphyle*, which is found in the summer. Barnes and McDunnough (1914) have been quite logical, therefore, in restricting this name for the spring or autumn form of *eriphyle* in southern British Columbia, even though there are no type specimens. The type locality of *eriphyle* (Lake Lahache) is close enough to Kaslo so that the populations at the two places may be considered as of the same subspecies. *Kootenai* Cockle, therefore, must fall as a synonym of *eriphyle* if environmental modifications of the phenotype are not to have Latin designations. Some authors (Barnes and McDunnough, 1914, Gibson, 1920, Clark, 1941) have used *kootenai* in the sense of a subspecies for the far Northwest populations. Though it is true that the spring form from southern British Columbia is quite similar to the far northern material of the summer and only generation, they are not identical and the name should not be used for this purpose.

A race *Colias eurytheme alberta* was described by Bowman (1942) from Alberta, Canada. An examination of a paratype in the Los Angeles Museum indicates that the specimen is of a race of the *christina-astraea*-etc. complex with more orange pigment than others of that group but not a race of *chrysotheme*. Therefore, the name was omitted from the synonymy above.

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Footnote

*Asterisk denotes that reference was not checked in the original.

Genetic Data on the two Races of *Colias chrysotheme* in North America and on a white Form occurring in each.

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Investigations on the population structure of *Colias chrysotheme* in North America have been made with a view towards determining the genetic and ecologic relationships of the three color phases existing in the wild, namely, the orange, the yellow, and the white.

A deduction derived from distributional data alone has been formulated viz that the color phases of butterflies are purely expressions of a different genetic adaptation to the environment (Hovanitz, '40, '41). According to this hypothesis, the colors are by-products of the adaptation, perhaps being alternative metabolic materials derived from the varying growth processes in turn under genetic control.

The present physiological and genetic data on the color phases of *Colias chrysotheme* suggest that the earlier view is essentially a correct one.

Historical Orientation

At the time that this work on the three color phases was initiated in 1940, a geographical part of the yellow form was considered by many authors to be a species separate from the orange form. Another geographical part was considered to be merely a seasonal form or alternate form of the orange species. Gerould ('23) had reported crosses made between the former two "species". He has since given more complete data on these results ('41, MS).

Edwards ('68-'93) first bred yellow forms from orange females and orange forms from yellow females; the interpretation he obtained from this work has had a profound influence on the taxonomic standing of the forms in later years and accounts in part for the present confused nature of the nomenclature.

Gerould ('23) has given the results of his crosses involving the inheritance of the white form in the yellow "species" (philodice) and the orange (eurytheme).

In this paper, these data are compared with additional data derived from new material. It has been concluded that the orange and yellow "phases" of Colias chrysotheme are throughout their entire range separable equally as genetic complexes which do not break down in crosses with the other type. Therefore, they have been designated as the orange-race and yellow-race respectively (Hovanitz, '42, '43a).

The genetic results presented here confirm the essential postulates of Gerould that the white phase is controlled by a dominant sex-limited gene which segregates with the normal orange or yellow phase.

The Interracial Crosses

The essential visible difference between the yellow and orange races lies in the orange suffusion which is ~~on~~ in the one and not the other on the upper surface of the fore wing. Though the color of the hind wing (upper side) cell spot, the color of the under side fore wing and the color of the upper side hind wing may vary in unison with the color of the fore wing upper side, in some races there may be a difference only in the latter. This is true, particularly for the females. A number of non-visible physiological differences have been found to be present between these races but owing to the difficulty of determination in individual cases, mention of these will be made later.

An arbitrary scale of from (1) to (10) was arranged, giving at (1) a light yellow color with no suffusion of orange and at (10) a full orange suffusion ~~on~~ the fore wing upper side ~~wings~~. The scale is useful mainly for comparing butterflies bred at uniform environmental conditions or at least those which appear to have had fairly optimum conditions. The reason for this is that the amount of orange pigment is directly changeable according to the environmental conditions, (Hovanitz '43b).

Gerould (MS) has used a tint photometer with which the degree of red in the wing pigment was obtained in a standard area near the base of the fore wing. His units range from 0.1 for a light yellow butterfly to above 5.6 for the orange. Approximate congruence can be obtained with Gerould's values by means of the following table. This is based upon some individuals sent him for determination and later compared with the present standard series. For the purposes of this paper, when Gerould's data have been mentioned his values have been translated into these standards.

Present grade	Gerould's grade
1	0.1-0.7
2	0.7-1.0
3	1.0-1.3
4	1.3-1.7
5	1.7-2.0
6	2.0-2.2
7	2.2-2.9
8	2.9-3.4
9	3.4-4.0
10	4.0-5.6 +

The accompanying table (table 1) shows the results of crosses between the orange and yellow races.

The segregations show that the factors controlling the orange coloration are multiple factor ones. The F_1 is normally exactly in between the two parental types. The F_2 present a wide range of variation nearly covering the range from 1 to 10. The backcrosses cover the grades between the parental types and, perhaps due to environmental

TABLE 1

Yellow X Orange racial crosses

The letters G and H refer to the source of the data, that is, Gerould or Hovanitz.

In parents, when grades are not given, yellow means grades 1 to 2 and orange from 7-10.

				GRADES											
	Source of Parents		sex	1	2	3	4	5	6	7	8	9	10	w	total
F1 A	Tempe, Ariz. orange X Hanover, N.H. yellow	G	male						2	24	3				72
			female						15	19	3			36	71
F1 B	Hanover, N.H. yellow X Tempe, Ariz. orange	G	male					8	12	6	2				122
			female					1	1	19	3			0	40
F1 C	"	G	male					4	9	30	2				45
			female							4	5			7	16
F1 D	Tempe, Arix. orange X Hanover, N.H. yellow	G	male			3	7	8	0	4					20
			female			1	4	4	0	1				24	34
F1 E	Round Valley yellow X Round Valley orange	H	male					2	13						15
			female												0
F1 F	Tempe, Ariz. orange X Hanover, N.H. yellow	G	male												1
			female			6	3	2							11
F1 G	"	G	male				14	11							25
			female											3	8
F1 H	Round Valley yellow X Round Valley orange	H	male					9							9
			female												
F2 A	Grade 7 from F1 B X Grade 8 from F1 B	G	male		2	0	0	3	0	2	1				6
			feamle		1	1	2	0	0	1				0	6
F2 B	Grade 5 Or 6 from F1 B X same	G	male		1	3	1	2	5	1	0	4			12
			female	1	2	1	3	1	2	1				0	22
F2 C	Grade 6 from F1 X Grade 6 from F1 A	G	male	3	3	4	2	1							13
			female				3							3	6
BC A	Grade 7 from F1 B X Hanover, N.H. yellow	G	male	7	2	0	3	5	3						24
			female	2	5	3	16	7	3					0	36
BC B	Intermediate X orange from Tempw, Ariz.	G	male			2	23	7	7	8	2	1			62
			female											0	29
BC C	Tempe, Ariz. w X Grade 7 from F1 A	G	male			2	5	2	4	11	4	2			33
			female											39	39
BC D	"	G	male			3	7	3	4	17	5	1			36
			female			0	4	3	0	4	0	2		15	28
BC E	Rio Hondo w X Grade 5 from F1 E	H	male							1	0	1	6		8
			female							2	4	1		5	12
BC F	Rio Hondo w X Grade 6 from F1 E	H	male						1	0	7	3	19		30
			female							2	5		1	3	11

shifting, somewhat beyond them. The broods of Gerould were subject to the outside environment at Hanover, N.H. and show seasonal changes in the phenotype. The summer broods show more orange than the autumn ones. The wide range in the F_1 variability (from 3-7 or from 5-8) is certainly due to the changeable environmental effect. The shift in F_1 mean from grade 4 in two cases to 7 in other cases is due to seasonal climatic effects. The F_2 segregations likewise show a seasonal shift in the mean from grades 3 or 4 to 5 or 6. The greater variability of the F_2 is expected.

The backcrosses are all late season broods and would show the effects of cold on the phenotype. Pertinent here is the expected reduction in orange. The crosses F_1 x orange parent and the reciprocal all show a shift toward yellow beyond the expected F_1 grade. The color plates of Gerould (MS) show that this is phenotypic. The segregation of the crosses by Hovanitz grown at near optimum does not show this shift in phenotype.

The lack of F_3 crosses is apparently not so much due to sterility in the butterflies as to the fact that stocks are usually lost during the winter at Hanover or original difficulties with food at Pasadena. A brood was obtained from two individuals which had arisen from a backcross. The backcross individuals are therefore fertile.

Tests of sexual selection between the yellow and orange race individuals, which have been made, have been found to have little value. This is due to the difficulties arising from the larval diet. Great difficulties had been found in getting orange X yellow matings and likewise even yellow X yellow. Orange X orange (including the white female) took place readily. This will be discussed under larval-diet sterility and sexual apathy. In the crosses made at Pasadena, no sterility has been found between yellow race and orange race individuals which cannot be traced to larval diet difficulties.

Sterility and Sexual Apathy Induced by Larval Diet

The breeding of the yellow and orange races of *Colias chrysotheme* in North America has shown that the two races differ in their food requirements. Sterility, sexual apathy, high mortality and phenotypic alterations are the result of interchanging the normal food of the two races.

Owing to the fact that females are often not too particular about the leguminous plants upon which they lay their eggs, a long list of larval food plants has accumulated in the literature. This list is not very different for the two races. Caterpillars presumed to be of either race (they are indistinguishable) have been taken on most plants on the list. In some cases larvae have been bred on the plant.

The recorded larval food plants of the orange race in the wild are as follows: alfalfa (*Medicago sativa*, introduced), *Trifolium* (including *repens*, *pratense*, etc. which are introduced and native clovers), Lupines (*Lupinus* sp.), vetches (*Vicia* sp.), loco-weed (*Astragalus* sp.), sweet white clover (*Melilotus alba* and other species), *Lotus* (Hosackia) *americana* and other species, various grasses.[1]

The author has observed in the wild the laying of eggs by the orange race on alfalfa, on white clover (*T. repens*) and native clovers, and on *Astragalus* sp., but not on red clover (*T. pratense*). Eggs and larvae have been taken from these same plants. Adults have been bred on alfalfa and on red clover. The adults were not fertile in the latter case.

Observations of oviposition were made at Round Valley in a field of mixed red clover and alfalfa of about equal proportions and height (1½ feet high). More than ten laying orange females were observed attaching their eggs onto the dorsal surface of alfalfa leaves, wholly ignoring for this purpose, the red clover in the field. Each of these females ~~were~~ was under observation for five minutes or more and some eggs were collected. At the same time five yellow females were observed laying on the red clover in the same field. It is not known if, when red clover alone is present in a field, the orange females will lay upon it. Scudder ('89) says that the orange females ignore red clover. Caged females of the orange type will lay upon red clover in the laboratory with no difficulty--the eggs being laid on the dorsal surface of the leaves. This observation is factual though contrary to statements of others that the hairiness of the under side of the leaves prevents the eggs from being attached.

Clark('32) records eggs being laid by this species (*eurytheme*) on red clover and on sweet clover (Melilotus). However, according to the view point at that time, the large, angular yellow butterflies present in the vicinity of Washington, D.C. were classifiable as *eurytheme* (or the orange form). It is now believed that the yellow phase in that locality represents the yellow race altered by repeated intercrossing with the orange. Therefore, it may have been yellow females which had laid the above eggs.

Larvae and pupae of the orange form bred on red clover are modified in coloration as compared with the same bred on alfalfa. The former have a bluish color to the blood, absent in the latter. The same difference has been noted in the yellow race but is not so striking. The effect of this color change of the blood is to give the larvae and pupae a blue-green color, as compared with a yellow-green color. The dung of the larvae of the former is also bluish and darker. It quite resembles the dried leaves of the red clover itself and is apparently derived directly from the food plant.

The listed food plants of the yellow race are the same as the orange race but in addition, the following: *Amorpha* (indigo; this is the normal food plant of Colias caesonia-eurydice), Baptisia tinctoria, Pisum sativa (cultivated pea), Cytisus (Scotch Broom), Caragana. The latter three are introduced from Eurasia.

The yellow form from New England, Colorado, and Kansas has been bred fertile on white clover (T. repens) by Gerould. From California (Mono Lake and Round Valley), yellow have been bred by the author fertile on red clover (T. pratense), on alfalfa and red clover in part, and alfalfa and white clover in part. In one case a fertile mating was made between a yellow male and a yellow female (Round Valley stock) bred wholly on alfalfa from which young larvae were obtained. They all died on alfalfa in the first instar. There is a possibility that had these larvae been on clover, they might have survived. Several stocks derived from New Hampshire wild yellow females have been bred with difficulty on alfalfa (at Pasadena). Some of these have died in various larval stages--others have nearly always gone through to the imago but with phenotypic alterations. Of 4 matings made between such individuals, one female laid fertile eggs. Larvae from this set died in the first instar on alfalfa. The "yellow" individuals bred on alfalfa are usually of a phenotypically reduced sort. They are much smaller and have a "spring type" of pigmentation (Hovanitz, '43b).

Larvae of the yellow races bred on alfalfa through several instars generally have a "sick" appearance. They have little vitality, their skin is wrinkled and their color is a lighter-than-normal green. In one case where several of these "sick" larvae in the last instar were obviously not far from the point of death, they were removed from the alfalfa and placed on white clover. Four out of seven apparently recovered enough to pupate. One female imago was obtained. She was mated to an orange male bred on alfalfa and laid fertile F_1 hybrid eggs. Seven yellow males and nine females of the same brood bred wholly on alfalfa would not mate with their kind nor with fertile "orange" individuals (reciprocal crosses). The one yellow female referred to above could not be mated to any of her yellow brothers bred on alfalfa though the attempt was made for several days before introducing an orange male into the same cage. This female was phenotypically also the most nearly full-grown of all the individuals of that brood.

The F_1 intermediates are apparently fertile on both alfalfa and white clover, at least some individuals. Gerould ('23 and MS) bred his F_1 on white clover and though some difficulty was met in getting copulations with some individuals, many were fertile. All Gerould's strains of the yellow race were bred on white clover; the orange were partly on alfalfa (personal communication).

The F_1 of Round Valley stock ~~were~~ was bred on alfalfa (parents were from alfalfa and red clover). At least two of these F_1 were fertile. In another F_1 cross (from yellow ♀ on alfalfa and white clover X orange ♂ on alfalfa), the adults seem to be normal. The eggs were fertile and larvae resulted. The first third of the larvae (about 15) were grown on alfalfa. The second third were removed from alfalfa immediately after hatching from the egg to white clover. They all died on the white clover in 4-5 days in their second larval instar. The third portion of eggs have grown to the imago on alfalfa.

The yellow female parent of the last F_1 discussed was the only female of her brood (about 10) which would mate with either yellow males (bred on alfalfa) or with orange males (bred on alfalfa). The others had a complete diet of alfalfa. This one female is the individual discussed just above which was partly bred on white clover.

The sexual apathy and sterility of yellow-race individuals bred on alfalfa may be illustrated by the following:

(a) Caged 5 white and 5 orange Garvey orange-race ♀♀ with

7 yellow-race (Round Valley) ♂♂. All were bred on alfalfa. In four days with excellent conditions no matings occurred. Orange race copulations can be expected to occur within a few hours, normally. The females were sexually very active but the males were not.

(b) Replaced the above females by sisters but in two days there were still no copulations.

(c) Caged 3 yellow sisters bred on alfalfa with 4 yellow males also bred on alfalfa (brothers of above). No copulations and little sex play.

(d) Caged yellow sister of above (from alfalfa) with Garvey (orange race ♂, brother of above ♀♀). Some sex play, especially by the male. Female was once noticed in typical mating pose with abdomen curved toward male and with wings spread. Contact was made with male but no copulation.

(e) Single pair mating of same individual male in (d) with an orange sister occurred in one hour after exposure.

(f) The 10 females in (a) were exposed to four brothers (orange). and three copulations occurred in the same day, 2 white females and one orange female.

Similar mating difficulties have been found with F_2 and backcross intermediates. The yellow half (grades 1 to 5) of the segregation when bred on alfalfa are sterile or impossible to mate. This suggests that the diet necessity is directly correlated with the yellow race and cannot be separated from it.

The desire or lack of desire to mate is not sterility. However, 3 crosses (copulations) made between yellow ♀♀ and yellow ♂♂ from the grades 1-4 of an apparently F_2 segregation (bred on alfalfa) produced a few eggs. The eggs laid did not start development. Crosses made between grades 6-10 and white ♀♀ of the same brood were fertile (9 crosses made) and progeny were fertile.

Sterility was noticed in two crosses made between New Hampshire yellows bred on alfalfa though fertile eggs were laid in a third case. The larvae died on alfalfa immediately after hatching.

Crosses were made between yellows arisen from a single pair mating, half of which were bred on alfalfa and half on red clover. Copulations could not be induced in individuals from alfalfa but several were made between those bred on red clover. Inter-strain (food strain) copulations were difficult as only one from several individuals occurred. No eggs were obtained. Interracial crosses were attempted with orange-race individuals bred on alfalfa. Combinations were made as follows (all individuals from these two strains unless noted):

A. yellow ♀♀(alfalfa) with orange ♂♂(alfalfa)

B. yellow ♀♀(red clover) with orange ♂♂(alfalfa)

C. orange ♀♀ (alfalfa) with yellow ♂ (alfalfa)

D. yellow ♀♀ (alfalfa but different stock) with orange (alfalfa).

Two copulations were obtained after two to three days:

yellow ♀ (red clover) X orange ♂ (alfalfa)

yellow ♀ (alfalfa from D) X orange ♂ (alfalfa).

Eggs from the former were fertile, larvae were obtained and F_1 bred on alfalfa. Few eggs from the second cross were obtained and these failed to start development (that is, remained yellow or white and then dried up).

Possible sexual selection of F_1 male hybrids against yellow females bred on alfalfa is ~~perhaps~~ illustrated by the following:

5 F_1 grade 5 and 6 intermediates (bred on alfalfa) were placed with 2 yellow females bred on alfalfa and three white females (orange race) bred on alfalfa. Two of the males mated the first day with two of the white orange-race females. The remaining three males, two

yellow females and one white orange-race female were kept together for five days more but with no further copulations. After the first day, the mated males were re-introduced with the remaining females but with no further copulations. The two white females laid fertile eggs (backcross).

The existence of yellow 'sports' or aberrations within orange-race populations (Hovanitz '43c) indicates that the orange pigment can be eliminated in some cases in a way separate from that differentiating the races. It has been shown that there is a series of genes or alleles controlling orange pigmentation in pure orange-race populations (Hovanitz, '43c). These are arranged in such a way as to give a continuous series from very orange butterflies to those completely lacking it. The latter are exceedingly rare in wild populations. The progeny of a yellow female of this sort bred on alfalfa were found to be fully fertile. So the food plant preference which is correlated with the color difference in the yellow-race is not so correlated in the yellow 'sports' of the orange-race.

<u>Summary</u>: It seems clear that the yellow race is sterile and sexually inactive when the larvae are bred on alfalfa, but not on white or red clover. The same is true for the yellow end of the F_2 and backcross segregations. Much of the sterility and sexual apathy apparently obtained in these crosses (such as by Gerould) may be related entirely to diet difficulties.

The orange race in one trial was sterile on red clover. Since it is not attracted to it in the wild as well, perhaps red clover is not a sufficient food for this race.

The yellow race is fertile and well attracted to both red and white clovers. The orange race has not been tried in the laboratory entirely on white clover but as the orange half of F_2 and backcross segregations grown on white clover by Gerould have been at least partly fertile, it may be assumed that this food plant is satisfactory. Orange females lay on white clover in the wild.

Yellow 'sports' in orange populations have the orange-race food preference.

Diapause

The yellow race during the middle of the larval stage is able to go into a diapause. This normally does not take place unless instigated by some environmental condition. Low temperatures, dried or frost-bitten clover leaves, the wrong food plant (such as alfalfa) and perhaps other things will incite the larvae to stop feeding and become dormant. This is true for larvae of New Hampshire yellow as well as for those from California and Colorado.

The orange race larva dies when conditions become untenable rather than becoming dormant. Cool temperatures which will induce diapause in the yellow race (0° C. to 10° C.) will merely slow up the development of the orange race. This fact was noticed by Edwards ('68-'93) but the significance was not apparent then. Gerould (MS) has mentioned this diapause in his yellow material also.

Parts of intermediate broods in which grades 1-5 individuals are expected will go into diapause when bred on alfalfa just as yellow-race stocks do. However, there is no knowledge that F_1 will do so. Orange strains derived from the grades 6-10 of these broods have shown no diapause tendencies when bred on alfalfa. Apparently the diapause is correlated directly with the yellow color and the food preference. These characters do not seem to be interchangeable between the races.

However, the existence of yellow 'sports' or aberrations within orange-race pure populations (see page 11) indicates that the diapause may be separable from the color difference. The eggs and larvae from one such individual (Grade 2) were bred out-of-doors (6° C. night to 20° C. day) on alfalfa in the cold of autumn. Under the same conditions, the eggs and larvae of several yellow females from the yellow-race population at Round Valley were bred. The 2nd instar larvae of the latter went into diapause on and under the leaves of the red clover whereas the former continued to grow to pupation and were partially killed by frost. This illustrates that the orange pigment can be eliminated without the necessity of introducing the controller of the diapause. This does not seem to occur in the yellow-race but only in the orange.

Intermediates in Wild Populations

Considerable intermating occurs between the orange and yellow races where populations of each exist in the same place. The more intermixed the food plants of each race are, the greater the hybridization between the two. In fields where alfalfa, red and white clover are commingled, the amount of intercrossing is very high. As females will generally mate immediately upon eclosion and rarely after the first copulation, the males which are present in the immediate vicinity of the newly eclosed female will be more likely to mate with her than males in adjacent fields. This would be true despite the flying of the females into adjacent fields. The important part of the intermixing of the adults must be the transfer of sperm from one race to the other. No amount of commingling after the mating period could have any effect on the genetic structure of the races.

Intermediates between the yellow and orange races have been found everywhere that the two races occur more or less together. Usually the habitats occupied by the races are different. An alfalfa field is usually a pure alfalfa field and can support only the orange race. Red clover fields are often pure red clover fields and can support only the yellow race. Astragalus, Vicia, etc. prefer dryer habitats than most of the clovers and microhabitat differences will then arise between the races. It is possible that prior to the growing of clover and alfalfa together as a hay crop, there may not have been a great deal of intercrossing between the races. In the 1880's, however, Edwards ('84) bred orange specimens from yellow wild Colorado females and yellow specimens from orange wild Colorado females. He makes no mention of intermediates and it is possible that these were either present or had died as larvae by reason of their lower viability. Table 2 shows the results of the breeding from wild females by Edwards.

The material was bred on "clover", probably white clover, so the diet would have been satisfactory for either race.

Records of copulated pairs of the orange and yellow races are very rare. Most collectors have seen orange X orange, white X orange, yellow X yellow and white X yellow pairs, but few have seen orange X yellow or the reciprocal. Edwards ('69-'93) gives the only record known in the literature of the 19th century: yellow ♀ X orange ♂ in copulation in Illinois. At that time this locality was as far east as the orange race could be found in fair numbers. Clark (MS) notes that yellow X orange and reciprocal crosses were common at the time of the first influx of orange race into the vicinity of Virginia but are no longer so.

Copulated pairs of intermediate and parental types have been taken at Mono Lake and Round Valley as follows:

♀ ♂
yellow X orange
orange X orange
white X orange
grade 6 X orange

♀ ♂
yellow X yellow
yellow X grade 6

The relative frequency of the types means little as there is reason to believe that more yellow females <u>in the mating period</u> are

Table 2.- The nineteenth century records of Edwards on intercrossing between the orange and yellow races in Colorado.

Parents	yellow	orange	white
6 yellow ♀♀	97	0	0
1 yellow ♀	0	12	3
2 yellow ♀♀	25	5	0
1 orange ♀	3	5	0

present at these localities than orange females.

Persons well acquainted with populations of this butterfly in areas where both races occur have not failed to note the blending of the characters of each. Elrod ('06) finds that in Montana there is no complete separation of the yellow form from the orange since they blend one into the other. Klots ('30) finds the same true for Wyoming. Each of these authors has, however, found that there are definite broods of one race occurring in certain locations or seasons. Elrod finds that the yellow race is more restricted in distribution to certain areas while the orange is everywhere. J.C. Hopfinger (personal communication) has noted the same for north-central Washington. Apparently, a similar situation is true for Kansas (Field, '39). The results of a complete analysis of populations at Mono Lake and Round Valley, California shows the same to be true (Hovanitz '43d).

Intermediates have been obtained in the wild from as far north as British Columbia and at least as far south as California, New Mexico and Georgia. They probably occur in Mexico as well. They are very common in eastern California and Nevada (Mono Lake, Round Valley, Honey Lake Valley, Modoc Co., Carson Valley), Great Salt Lake Valley (Utah), Montana, Idaho, Colorado, New Mexico, Kansas and the vicinity of Washington, D.C.

A very early record for New England of an "orange philodice" is by Scudder ('89). This specimen is probably an intermediate between the races and was taken in Bangor, Maine.

Breeding from wild intermediates:

The progenies of many wild intermediate females have been bred on alfalfa, or at least the attempt has been made to do so. Many of these did not get far beyond the early larval stages, possibly due to diet difficulties or lethal recombinations of genes (see egg counts later). The main conclusions to be derived from these broods are

(1) that the wild intermediates may be F_1, F_2, backcross, F_3, F_4, F_n individuals, (2) that the wild intermediates which have laid any eggs at all have laid mostly fertile ones, (3) that the phenotype of the intermediate is true to its genotype, and (4) that the progeny give a range of variation as expected had the female mated with a given hypothetical male.

Table three gives the grades of progeny bred from wild intermediate females. The numbers are small due to the fact that intermediates between grades 1 and 6 were usually selected as parents. As only alfalfa was used as food, the mortality is very high. Likewise, the yellow end of the segregation would be expected to have a higher mortality than the orange end and the progeny would be expected to be sterile due to diet. The unknown males were probably of the yellow race judging from the segregation in the progeny and the fact that most males at Round Valley are yellow. The white female from Mono Lake was probably an F_1 intermediate; her mate must have been that too in order to have given a range of intermediates and parental types such as that shown. The skewness of her curve (high on the orange side and low in the center) might be accounted for by sperm from several males but the 13:56 ratio of white: orange-yellow could not be. It is more likely that half of the expected white females were of the yellow type and had died before the imago stage. The skewness of the curve is also best answered by high mortality on the yellow side; the latter individuals which were present were sterile.

Genetic Data on the White Form

In the wild, individuals have been found with all yellow and orange pigment replaced by white, or various degrees of substitution can be found. White individuals exist most commonly in the female sex but occasionally males have also been taken. A clear-cut segregation

TABLE 2

TABLE Segregation in progeny of wild intermediate females
or females which gave intermediate progeny; from Mono Lake
and Round Valley, Calif.

Source of parents		sex	GRADES 1	2	3	4	5	6	7	8	9	10	w	total
Grade 1 R.V. X Grade 6 R.V. (wild cop.)	H	male	2	0	0	1	1							4
		female		2	0	0	2						0	4
Grade 4 R.V. X ? R.V. (prob. yellow)	H	male	4	1	1	2	1							9
		female	1	0	2	2	0	1					0	6
Grade 6 R.V. X ? R.V. "	H	male	1	0	3	1	2	1						8
		female	4	2	1	3	1	2					0	13
Grade 6 R.V. X ? R.V.	H	male	1											1
		female											0	0
White (2-2) M.L. X ? M.L. "	H	male	6	5	0	0	2	6	4	10	16	21		68
		female	3	4	2	0	1	5	8	11	6	3	13	56
White R.V. X ? R.V. "	H	male	2	0	0	0	0	0	0	0	0	5		7
		female											0	0
Grade 4 R.V. X ? R.V. "	H	male	6	2	1	2	1							12
		female	2	3	2	0	1						0	8
Grade 5-6 R.V. X Grade 1-2 M.L.	H	male	2										0	2
		female											0	0

into white versus yellow or orange usually makes a classification of these forms quite easy. However, there is considerable fluctuation within each type and some individuals exist which bridge the gap. The latter individuals have been observed only in the wild.

That the common white female variety of each of the yellow and orange races was a genetic variant was ~~really~~ first noted by W.H. Edwards ('68-'93) though he did not understand the significance of it. Edwards bred both yellow and white progeny from a yellow female. The numbers in the brood were, however, too small to give much genetic information (see table 2.) ~~on F_1 ratios from wild females~~).

Mr. Thomas E. Bean in 1891 (see Gerould, '23) bred the progeny of yellow and white wild females of Colias christina from Alberta, Canada. This species shows a much higher degree of intermediacy between the colored forms than do the races of Colias chrysotheme. For the most part, however, the progeny could be classified as white or yellow. Except for the progeny of one female of an intermediate type between orange, yellow and white which gave 16 progeny of various grades of the three colors, the results seem comparable to the genetic results in the chrysotheme group to be discussed later.

Frohawk ('01) bred progeny in the ratio of 110 w ♀♀: 125 or ♀♀ from 4 white wild females of Colias croceus = edusa (European species). This is probably a 1:1 ratio.

Main and Harrison ('05) bred progeny from a wild white female of crocea in the ratio 52 white ♀♀: 19 orange ♀♀ (3:1 ratio).

Pieszczek ('17) has bred butterflies from white females of Colias myrmidone of Europe and Asia Minor where the frequency of whites varies from more than 50% to close to 0%.

2 white ♀♀ (Upper Styria): 30 w : 0 or = 1:0 ratio
1 white ♀ " " : 18 w : 17 or = 1:1 ratio

(Majority of females in Upper Styria are white though yellows, oranges, and whites are common in the populations).

5 white ♀♀ Graz = 23 w : 19 red (orange) = 1:1 ratio
(White females at Graz are fairly rare. Population count of 33 red; 7 intermediate; 11 white.)

Gerould ('11, '23) has studied the inheritance of a genetic factor controlling white wing color in females of *Colias philodice* (yellow from New Hampshire) and *Colias eurytheme* (orange from Arizona). In the earlier paper, the author showed that the gene was dominant and he postulated homozygous lethality due to the difficulty of getting 3:1 ratios (nearer 2:1 was the result). Later, this was assumed to be due to the existence of an occasionally linked lethal recessive factor for broods were obtained having 3:1 ratios as well as 100% white progeny.

Likewise, unexpected ratios, such as 100% white females from a supposedly heterozygote X heterozygote led Gerould to postulate a lethal factor associated with the recessive factor for yellow. The data of Goldschmidt and Fischer ('22) on a similar sex-limited gene in *Argynnis paphia* of Europe gave similar results; it was concluded that the dominant gene was usually linked with a recessive lethal. The present author believes that the evidence needs to be reconsidered and that the reason for these aberrant results may lie in the effect of modifying factors.

From ratios obtained from wild females alone, it is clear that the gene controlling white female color is dominant, in both the orange and yellow races. This conclusion has been arrived at by Gerould ('23). White females have never failed to produce at least some white female progeny when the total number of female progeny was large. Only one wild female of this species has yet produced a 100% white female brood which is statistically significant and this one from a locality where the white frequency was 71 to 74% of the females in the population.

The two white females of the European Colias myrmidone of Europe produced all white broods; these also came from a population having a high white frequency.

It can be assumed fairly safely that most wild females have mated but once. However, old females have been found in copulation. Old females have been mated more than once in the laboratory, but only when the supply of sperm has been exhausted; then the females have exhausted their supply of eggs.

Males are apparently not affected phenotypically by the genetic controller of the white color in these females. However, the existence of very rare white males in wild populations suggests that there are genes which do control this character or a similar one on a bisexual basis. Except for one case which will be brought up later, no white males have appeared in any of the cultures. This one exceptional case is obviously due to a different recessive gene with a much more drastic effect than the dominant one.

Though the males are indistinguishable phenotypically, they apparently carry the gene for female whiteness in the same manner as do the females. The following genotypes should, therefore, appear in wild populations in various frequencies providing that none are lethal, notwithstanding the fact that visibly the males are indistinguishable:

yellow or orange ♀♀	ww
white ♀♀	Ww, WW
yellow or orange ♂♂	ww, Ww, WW

Individuals giving genetic results expected of all the above genotypes have been tested in either the yellow or orange races with the one possible exception of the homozygous dominant male. The difficulties in making the desirable tests of individuals have their foundation in the laborious work involved in breeding the material. Without tremendous facilities, it is impossible to carry the larvae of many crosses through at any one time. Ten full grown larvae will consume a two foot high alfalfa plant in 1 to 2 days at 25° C. Also, since the genotype of a male cannot be told phenotypically, one must partly rely upon a lucky choice for an individual.

Without a narrative of minute detail on all the crosses made in testing the genotypes of the above forms, it will be impossible to describe the pedigrees of all the crosses made. Of course, this will eliminate desirable detail, especially where the small ratios are concerned. The latter have been summarized in the places where they seemed to fit best according to their pedigree.

Knowledge if the existence of all the various combinations of genes is desirable from the point of view of the genetics of wild populations. For that reason, the ratios from wild females are compared with those from laboratory ratios. The wild females from which these ratios have been derived have come from many geographical sources, namely, Hanover, N.H. (yellow-race), Pueblo, Colorado (yellow- and orange-races), Centennial, Wyoming (yellow-race), Tolland, Colorado (yellow- and orange-races), Manhattan, Kansas (yellow- and orange-races), Kearney, Nebraska (yellow-race), Round Valley, California (yellow- and orange-races), Mono Lake, California (yellow- and orange-races), Tempe, Arizona (orange-race), Rio Hondo (near Los Angeles), California (orange-race), Garvey, California (orange-race), Westley, California (orange-race), Elsah, Illinois (orange-race), San Fernando Valley, California (orange race), Bouquet Canon, California (orange-race). Some data are from Gerould ('23), and Edwards ('69-'93); the remainder from cultures of the author. The data indicate that the genetics of the variations are comparable in all localities. For specific details on geographical distributions of the races and frequencies of the white forms, maps are given in another article (Hovanitz '43e).

Granting the dominance of the white allele, the ratios from yellow or orange wild females giving all orange or yellow female progeny ~~in table 4~~ are evidence of crosses in the wild involving a homozygous recessive female and a homozygous recessive male. These total 0 white: 505 yellow in 31 broods, 0 white: 248 orange in 18 broods, or an interracial total of 0 white: 753 yellow-orange in 49 broods. Laboratory crosses giving the same ratio total 0 white: 216 yellow in 10 broods, 0 white: 696 orange in 18 broods or a grand total of 0 white: 912 yellow-orange in 28 broods.

Ratios of 1:1 from orange or yellow females would suggest the cross recessive female X heterozygous male. Of wild ratios of this type there are but two in the yellow-race totaling 8 white: 11 yellow. In the orange-race there are several, 28 w: 18 or, 13 w: 17 or, 12 w: 10 or, 21 w: 18 or, 21 w: 29 or, 20 w: 23 or, 13 w: 15 or in a single brood ratios and 9 w: 13 or in 4 small broods, a total of 137 white: 143 orange females.

The lack of F_1 ratios from wild yellow or orange females giving much more than a 1:1 ratio may be taken as evidence against the existence of a yellow or orange heterozygote; were the latter in existence, 2:1 or 3:1 ratios would occur in the F_1 from wild material more frequently.

There are many small broods close to 1:1 or 0:1 but in which the male is known to have been a heterozygote. These have been included in the following totals for 1:1, 13 w: 14 y, 7 w: 5 y, 19 w: 19 y, 28 w: 28 y, 59 w: 76 y, 1 w: 2 y, 10 w: 12 or, 7 w: 14 or, 30 w: 14 or, 43 w: 35 or, 24 w: 20 or (in 6 broods), 5 w: 14 or, giving a total for the two races of 216 white: 246 yellow-orange.

These data show that all ratios obtained from yellow or orange females are either 0:1 or 1:1. The 30:14 ratio in one case may be significantly different or not. The history of this ratio is ~~as~~ given in table 4 (from Gerould '23, reorganized).

Males have, in all cultures which are normal, a greater chance of survival over the females due to their faster development. When culture conditions are poor, it is the females which suffer most. This always gives a higher frequency of males in the cultures. In the ~~above~~ brood B3 (table 4), the males are about half that which would normally be expected, whereas the other broods are normal. It would appear that either an error has been made in recording the results or that something has happened to the sex determination so that about half the normally expected males have become white females. Or possibly the unusual rare ratio has appeared as expected on statistical grounds.

Part of the data given above for the homozygous recessive female is equally applicable as evidence for a homozygous recessive male. All the 100% yellow or orange female broods must have had a homozygous recessive male parent.

Ratios obtained from white females which approximate 1:1 suggest that the male was homozygous recessive (and the female heterozygous). 1:1 ratios from wild white females of the <u>yellow race</u> are as follows,

22 w: 28 y
15 w: 13 y
13 w: 11 y
8 w: 17 y
28 w: 21 y
7 w: 16 y
30 w: 38 y (in 7 broods)

or a total of 124 white: 144 yellow. 1:1 ratios from wild white females of the orange race are as follows

29 w: 24 or
27 w: 20 or
13 w: 15 or
62 w: 53 or
16 w: 8 or
6 w: 13 or
20 w: 15 or
14 w: 10 or
16 w: 6 or
18 w: 21 or
32 w: 20 or
4 w: 0 or (in two broods)

Table 4.-- Pedigree involved in the 30: 14 ratio of Gerould.

	white♀♀	yellow♀♀	♂♂	Expected
A. Wild white ♀	15	13	30	1:1
B1. w♀ X y♂ from A	38	22	63	2:1 or 3:1
B2. w♀ X same y♂ as in B1	13	8	23	2:1 or 3:1
B3. y♀ X same y♂ as in B1	30	14	27	1:1
B4. y♀ X y♂ from A	19	19	46	1:1
B5. w♀ X y♂ same as in B4	3	3	4	2:1 or 3:1

or a total of 257 w: 205 or. This gives a grand total for the two races of 381 white: 349 yellow-orange.

Ratios from laboratory crosses involving a white female and where a 1:1 is expected are as follows,

28 w: 38 y
14 w: 10 y
65 w: 69 y
20 w: 13 y
6 w: 11 y
21 w: 17 y
13 w: 12 y
14 w: 9 y
8 w: 8 y
30 w: 35 y (in 10 broods)

or a total of 219 w: 222 y in 19 broods. These same ratios from orange-race white females are as follows,

16 w: 11 or
28 w: 29 or
9 w: 10 or
11 w: 11 or
6 w: 13 or
1 w: 13 or
27 w: 22 or
15 w: 13 or
43 w: 35 or
10 w: 12 or
20 w: 16 or
21 w: 19 or (in 7 broods)

or a total of 207 w: 204 orange in 18 broods. The interracial total is 426 white: 411 yellow-orange.

The evidence for the existence of the heterozygous white female has been partly covered above under homozygous recessive male X white female giving 1:1 ratio. No wild females representing crosses involving heterozygous white females and heterozygous males, giving ratios approximating 2:1 or 3:1, have been taken in the wild though such wild crosses must occur at a frequency varying directly with the gene frequency in the population.

Laboratory crosses giving such ratios or where those ratios are expected are as follows,

67 w: 36 y	
49 w: 25 y	
4 w: 0 y	
17 w: 0 y	14 w: 4 or
28 w: 0 y	9 w: 0 or
38 w: 22 y	39 w: 21 or
13 w: 8 y	83 w: 31 or
95 w: 28 y	34 w: 15 or
10 w: 2 y	37 w: 22 or
321 w:121 y	216 w: 83 or
(2.65 w: 1 y)	(2.60 w: 1 or)

The heterozygote X heterozygote cross is expected to give a 3:1 ratio where the homozygous dominant is viable but indistinguishable from the heterozygote. Instead, however, the ratios vary from 3:1 to 2:1 with an average of 2.65:1 in yellow and 2.60:1 in orange. These averages, of course, mean only that neither 2:1 or 3:1 ratios are exclusively obtained. The production of 2:1 or 3:1 ratios seems to run in certain families. This was true also in Fischer and Goldschmidt's ('21) work on <u>Argynnis</u> <u>paphia</u> and its melanic mutant where an average of 2.57:1 was obtained. Some families of the latter species gave 3:1, others 2:1 leading Goldschmidt to predict that a lethal recessive factor was associated with the dominant gene in certain cases and absent in others. In the more highly inbred strains, the 3:1 ratio was obtained whereas 2:1 was obtained in relatively slightly inbred or F_1 from wild strains. Gerould ('11) first assumed that the homozygous dominant was lethal on the basis of 2:1 ratios in three broods. Later ('23), he postulated a recessive lethal factor which was eliminated in certain strains because 3:1 and wholly white strains were later obtained between 1911 and 1923. He also postulated a recessive lethal factor linked with the recessive yellow gene due to some anomalous broods of 2:1

where 1:1 was expected. This history of one of these broods is illustrated in Table 5.

It is seen that the total of the ratios for all three white sisters mated to the same male is 103:82 though one of these white females gave 67:36. Yellow sisters of these white females gave with the same male a ratio of 0 w: 12 y ♀♀. The probability that the latter ratio is 1:1 is .0002, or quite unlikely. Therefore we may assume that the male is homozygous recessive. It should give a 1:1 ratio with heterozygous females. The ratio 103:82 has a standard error of 6.8. Since the deviation from the expected ratio is 10.5 (considerably less than 2 X the standard error), the difference need not be taken seriously.

In the specific case of the ratio 67:36, the standard error is 5.8 and the deviation from the 1:1 ratio is 15.5. The actual deviation is, therefore, just over 2 X the standard error. It may be significant. However, a linked lethal factor with recessive yellow is not necessary here.

All F_1 from orange or yellow females that give white progeny in a 1:1 ratio are evidence that yellow or orange males are heterozygous for the dominant gene. These are listed in the section on homozygous recessive females. Other ratios of 3:1, 2:1 and some 1:0 could also be due to a heterozygous male but are listed elsewhere.

A homozygous dominant white female should give 100% white female progeny with any male. There are several broods of small number which have all white females; actually a ratio of 18 w: 0 or has a probability of .0056 of being a 3 w: 1 or ratio. Despite this, some low ratios are listed here. Only one ~~wild~~ ratio of many all white females from a wild female is known. Of course the female might have been an heterozygote which had mated with a homozygous dominant male. The same is likewise true of the laboratory crosses; whether the male or female was homozygous is unknown. No all-white brood has yet been obtained from an orange or

Table 5.— Pedigree involved in a 2:1 ratio of Gerould.

		white♀♀	yellow♀♀	♂♂	expected
A.	wild white ♀	3	4	1	1:1, 3:1
B1.	w♀ from A X y♂ from A	28	38	64	1:1, 3:1
B2 .	w♀ from A X y♂ same as in B1	8	8	26	1:1
B3.	w♀ from A X y♂ same as in B1	67	36	77	1:1, 2:1
	total from 3 white ♀♀	103	82	167	1:1
B4.	y♀ from A X y♂ same as in B1	0	7	13	0:1
B5.	y♀ from A X y♂ same as in B1	0	5	3	0:1
	total from yellow ♀♀	0	12	16	0:1

yellow female (except possibly one); such a ratio would suggest a homozygous dominant male was involved.

19 w: 0 y
28 w: 0 y
24 w: 0 y
15 w: 0 y
10 w: 0 y
10 w: 0 y
21 w: 0 y
2 w: 0 y
2 w: 0 y
4 w: 0 y
Total= 135 w: 0 y

9 w: 0 or
25 w: 0 or
15 w: 0 or
12 w: 0 or
Total = 71 w: 0 or

This gives a grand total of 206 white: 0 yellow-orange. Broods of all-white females from wild females are: (1) from orange female - 11 w: 0 or; (2) from white females - 7 w: 0 or, 11 w: 0 or, 28 w: 0 or or a total of 46 white: 0 orange.

The accompanying pedigree (Table 6, reorganized from Gerould, '23) is given to indicate the possible effect of selection of modifying factors upon the lethality of the homozygous dominant. When the material is inbred, white females are apparently more abundant in the crosses than when outbred to wild material (where the white frequency is low).

So far we have been considering all the white females as phenotypically identical; but this is not true. Some are whiter than others, have the melanic pigmentation reduced, have the orange cell spot of the hind wing reduced to a pale yellowish color and have the yellow-orange pigment of the under side hind wing and apex fore wing exceedingly light in color. These differences are genetic. In view of the fact that there is no sharp delimitation between the lightly pigmented individuals and the darkly pigmented ones it was not possible to segregate them into

Table 6.- Pedigree showing the effect of source of the genetic strain upon ratios of white to yellow females (reorganized from Gerould).

	white ♀♀	yellow ♀♀	♂♂	expected
A. Wild white ♀ (Hanover, N.H.)	1	3	5	
Crosses from A.				
B1. w♀ X y♂	95	28	130	3:1
B2. y♀ X y♂ (same as B1)	28	28	77	1:1
B3. y♀ X y♂	59	76	146	1:1
B4. y♀ X y♂	0	118	127	0:1
Crosses from B3.				
C1. w♀ X y♂	14	10	20	1:1
Crosses from B1 (unless noted).				
D1. w♀ X y♂	24	0	17	1:0
D2. w♀ X y♂	2	0	2	
D3. y♀ X y♂ from B4	0	13	21	0:1
D4. w♀ X y♂	10	0	7	1:0
Crosses from D4.				
E1. w♀ X y♂	21	0	29	1:0
E2. w♀ X y♂ (same as E1)	4	0	5	1:0,3:1
E3. w♀ X y♂	2	0	8	1:0,3:1
Crosses from D1.				
F1. w♀ X y♂	1	0	1	
F2. w♀ X y♂ same as in F1	15	0	2	1:0
F3. w♀ X y♂	10	0	14	1:0,3:1
F4. w♀ X y♂ same as in F3	2	0	4	

(continued)

(Table 6 continued)

		white ♀♀	yellow ♀♀	♂♂	expected
Crosses from F2 (unless noted)					
G1.	w♀ X wild ♂	6	11	22	1:1
G2.	w♀ X wild ♂	65	69	217	1:1
G3.	w♀ X wild ♂	1	1	1	
G4.	w♀ X wild ♂	3	4	9	
Crosses from G2.					
H1.	w♀ X y♂	10	2	19	
H2 .	w♀ X y♂	5	6	16	
H3.	w♀ X y♂	49	25	80	2:1
H4.	w♀ X y♂	20	13	40	
H5.	w♀ X y♂	3	3	8	
H6.	w♀ X y♂	14	9	45	
H7.	w♀ X y♂	13	12	26	
H8.	w♀ X y♂	21	17	36	1:1
H9.	w♀ X y♂	28	0	30	1:0
	total	163	87	300	2;1
Crosses from H3.					
I1.	w♀ X y♂	0	2	1	
I2.	w♀ X y♂	19	0	10	1:0
I3.	y♀ X wild y♂	0	20	2	0:1
Cross from H5 and H7.					
J1.	y♀ from H5 X y♂ from H7	0	8	7	0:1

(Notes to follow under Table 6)

B1 is a definite 3:1 ratio rather than 2:1 as often occurs in material from a locality where the white frequency is low. B2 shows that the male used in B1 was heterozygous. The higher number of females of yellow color in B3 is hardly significant. B4 as well as B2 and B3 show that the yellow females are certainly homozygous recessive. The male in C1 is surely a heterozygote. D1 to D4 show that the males of B1 as well as the white females are homozygous or heterogygous dominant. The yellow female in D3 from B1 is definitely homozygous recessive. E1 to E3 show the existence of homozygous and heterozygous dominants in D4. F1 to F4 shows the same less definitely for D1. In G1 to G4 the introduction of wild males which have an 83% chance of being homozygous recessive has eliminated homozygous dominants. The intercrossing of heterozygotes in H1 to H9 has given at least one 2:1 ratio. The 28:0 ration in H9 is an enigma. The I2 ratio suggests that there were homozygous dominants in the 49:25 ratio of H3.

heterozygous and homozygous dominant on these characters alone.

A series of six standards was made of each of two of these characters, namely, darkness of orange pigment in (1) the hind wing cell spot and (2) the under side pigmentation. ~~These characters are directly correlated as shown by a correlation coefficient of~~ . A formula is used to designate any one individual with respect to grade of these characters, for example:

(1-1) = grade 1 in both characters.

(2-5) = grade 2 in cell spot pigmentation, grade 5 in under side pigmentation, etc.

The crosses shown in Table 7 were made in order to test the genotype of different white females of these types. A single male was used with several females since the phenotypes of the males are the same. The results show decidedly variant ratios are obtained from the different white females mated to a single male. Cross H. shows that the male chosen was a heterozygote. Crosses C and G suggest that the white females (3-3) and (2-1) were homozygotes. Cross E suggests that the female (5-5) was a heterozygote but that the homozygote white females did not appear in the progeny. However, the probability for a 2:1 ratio is .1131 and for a 3:1 ratio it is .0829, i.e., either is not unlikely. Cross F (5-5) and A (2-2) are the same as E.

More detailed and complete data are needed on the relation between the degree of coloration of these characters and the genotype of the individual. It may be that the whiter individuals are the homozygotes though the results above seem to indicate that this is not always true. A review of the percentage of whites and its relation to the minor characters in wild populations will be given elsewhere. There is no sharp distinction between these minor variations but rather a continuous range of variability. However, populations in which the white female is abundant have a higher percentage of those individuals having

Table 7.– Pedigree involving white females having different modifiers for white color. All the females listed have been mated to the same male (bred from wild pupa from the San Fernando Valley, September 23, 1942)

	♀ parent	white ♀♀	orange ♀♀
A.	white ♀ (2-2) bred from egg from Westley, Calif.	37	22
B.	white ♀ (1-1) same source as male, all died	--	--
C.	white ♀ (3-3) same source as male	15	0
D.	white ♀ (5-3) intermediate white-orange, all died	--	--
E.	white ♀ (5-5) same source as male	34	15
F.	white ♀ (5-5) same source as male	14	4
G.	white ♀ (2-1) same source as male	25	0
H.	orange ♀ (6-6) same source as male	10	12
I.	orange ♀ (2- 1) same source as male, all died	--	--

formulae running from 1-1 to 3-3 than populations where the frequency of whites is low. It may be that homozygotes are more viable under the supplementary action of other genes which, on the side, affect the color of the cell spot and under surface.

Table 8 ~~shows~~ gives a pedigree illustrating the shift of ratios from white to orange and the reciprocal depending upon the source of the material.

Parent A or its mate ~~might~~ may have been homozygous white or heterozygous white. Round Valley had more than 50% females white in the orange race at that time. The male and females chosen for A1 might also have been heterozygous or homozygous for dominant white. The progeny of A1, therefore, could have been homozygous white, heterozygous white or some males might have been homozygous recessive.

Parent B was a wild intermediate, having the anterior half of the fore wings white, the rest of the wings normal yellow-orange. It probably was genetically homozygous recessive and had mated with a similar male.

Parent C was a very pale yellow-orange female. It was for this reason that it was bred. Its F_1 and F_2 progeny were full orange, however. An 11:0 ratio obtained from it suggests that it might have mated with a homozygous dominant male. The probability that 11:0 could have been the ratio of a ww ♀ X Ww ♂ is .0005, not very likely but still possible. Its source (San Fernando Valley) is not the place where homozygous males are likely to be very common.

Culture D5 gives a ratio well within the expected for a 3:1 ratio (probability .0535); both males and females were probably heterozygotes.

Culture D6 gives a ratio which should be 1:1 but instead is 5:21 (probability of being 1:1 is .0010). The chances are not great that it is 1:1 but since the male was the same as in D5 and is there obviously a heterozygote we may assume that perhaps the high death rate of the

Table 8.-- Pedigree showing the shift of ratios from white to orange depending upon the strain used for the female. 2

		white ♀♀	orange ♀♀	♂♂	expected
A.	Wild white ♀ from Round Valley (3-3)	7	0	18	
A1.	white ♀ X or ♂ from A	12	0	+	
B.	Wild intermediate white-orange ♀ from San Fernando Valley	0	4	7	
C.	Wild orange ♀ from San Fernando Valley	11	0	16	
All from C unless otherwise noted					
D1.	white ♀ X or ♂				
D2.	white ♀ X or ♂				
D3.	white ♀ X or ♂				
D4.	white ♀ X or ♂				
D5.	white ♀ X or ♂ (same as in D1)	15	1	33	3:1
D6.	orange ♀ from B X or ♂ (same as in D1)	5	21	57	1:1
D7.	white ♀ from A1 X or ♂ (same as in D1)	29	0	35	1:0
D8.	white ♀ X or ♂ (same as in D1)				
D9.	white ♀ X or ♂ (same as in D1)				

cultures have created a selection for the orange females. It may be that in a given genic environment where modifiers have been selected for action with a particular gene, this gene may give one allele an advantage over the other. The environment, too, may select modifiers and thus allow a given allele to exist in a higher frequency in some places than in others. Correlated with this theory may be the probable death of homozygote dominants in some strains and their existence in others. Obviously, the problem needs study with a greater control of the genic environment as well as larger numbers of progeny brought to maturity and tested. It should be noted that the orange female parent of this culture is from a locality where white females are but 30% of the female population.

Culture D7 gives a ratio which is one hundred percent white. The male is the same heterozygote as in D5 and D6 but the female is from a strain producing in one generation 7 white females and 12 white females in the following. The female strain arose from a population where the white female frequency was more than 70%. The chances are great that the strain A1 had heterozygotes and homozygote dominants. The female of D7 must have been a homozygote unless the orange females expected were eliminated in the strong selection. Granting no selection, the 29:0 ratio has a probability less than .0001 of being a 2:1 ratio and of .0002 of being a 3:1 ratio.

It was in the progeny of the crosses (D) that the realization became firmly established that variations of the cell spot color and under side hind wing color of white females ~~was~~ were genetic. Though most of the material was discarded before a check against the standard series was made, the data given in Table 9 were obtained.

Apparently the white females from the orange parent, though by the same male, have decidedly a stronger orange cell spot and under side coloration. The white females from the white inbred parents have

Table 9.- The grades of colors due to modifying factors or different genotypes on white females from different colored parents. Pedigree of the crosses is given in table 8.

Cross	Parental color	Ratio	Average of grades	Number graded	Highest grade	Lowest grade
D5	white	15:1	1.65-1.75	8	3-3	1-1
D6	orange	5:21	5.00-5.00	2	5-5	5-5
D7	white	29:0	1.80-1.55	9	3-3	1-1

a very light spot and under side coloration. These data are also suggestive of a selection of modifiers for the better adaptability of the dominant gene in the genom as has just been discussed.

Ford ('40) in Abraxas grossulariata has found that the yellow and white varieties are under unifactorial control and that the heterozygotes are intermediate. However, he finds that by plus and minus selection, these intermediates can be shifted from a continuous distribution with one parental type to the other. The shift, he indicates, is by altering the genom rather than the gene itself. Similarly, it may be that the heterozygote and homozygote dominant white females in Colias are distinguishable by the formula (1-1 being full homozygotes and 6-6 or 5-5 being heterozygotes) but that the variability in the genom accounts for the continuity between them, the gap between the homozygous recessive (orange phenotype) and the heterozygote being too great to bridge except in a few populations.

Conclusions: The preceding data illustrate that in both the yellow and orange races the heredity of the white female form is similar. In both races, the expected 3:1 ratio may be reduced to 2:1 or some intermediate condition. Also there is a possibility that under a given gene environment one or the other of the homozygotes is at a disadvantage, thereby being selected out in a rigorous external environment. Apparently, the white gene is at its best in a gene environment such as that existing where the white gene is most abundant. The evidence suggests that any experiment designed to test the differential ability of the white vs. orange or yellow females to survive must be combined with an intensive study of modifier selection.

From the standpoint of genetics of wild populations, when comparing the gene frequency in different geographical regions, the different ability of one genotype to survive (as for example, homozygous dominant) may be different in different places. Since this is probably true in more cases than not, the study of wild gene frequencies must necessarily be much more complicated than in present generalized work on the subject.

Effect of Interracial Crosses on the Inheritance of the Dominant Gene for White

The question of homologies of genes between the orange and yellow races has not been attacked as in Drosophila (for review see Sturtevant and Novitzki, '41). However, the apparently normal segregation of the alleles in the interracial crosses suggests that the dominant white gene for female color is enough alike to be interchangeable in the races. The accompanying data on segregations are partially taken from the general tables of Gerould ('23) (Table 10).

Except for the one ratio of 13 white to 53 yellow and orange, ratios are normal. The abnormal ratio can be accounted for by differential mortality due to larval diet as discussed earlier.

The important point here is the fact that the white gene is transferred easily from one race to the other. Apparently, this is not true for all genes which differentiate the races. Therefore, by sufficient crossing between the races in wild populations the white gene frequency in the two races will reach an equilibrium at the same area.

The White Male

White males have been obtained in widely separated geographical regions:

Batavia, New York (1891) (probably yellow race)
Columbia, Missouri (orange or yellow race)
Alstead, New Hampshire (probably yellow race)
Finleyville, Pennsylvania (probably yellow race)
Sarver, Pennsylvania (probably yellow race)
"Western Pennsylvania" (probably yellow race)
"Michigan" (orange or yellow race)

Table 10.- Ratios involving dominant white for female color in interracial crosses, illustrating that the gene is segregated independently of the racial complex.

cross	♀ parent color	white ♀	yellow, inter., orange ♀♀	♂♂
Orange race X Yellow race	white	36	35	72
"	white	3	5	25
"	white	4	3	11
"	3 w♀♀ total	43	43	108
"	orange, yellow	0	11	1
"	orange, yellow	3	1	1
"	white	24	10	20
Orange race X F_1 intermediate	white	4	3	5
"	white	35	38	108
"	white	15	13	36
"	white	5	7	8
"	white	3	8	30
"	5 w♀♀ total	62	69	267
"	white	39	0	33
F_1 inter. X F_1 inter.	white	3	3	13
"	intermediate	0	6	6
"				
"	intermediate	0	12	22
F_3 inter. " F_3 inter.	intermediate	0	8	14
Yellow race X Orange race	yellow	0	40	122
"	yellow	7	9	45
F_1 inter. X Yellow race	intermediate	0	36	24
Yellow race X grade 6 inter.	yellow	0	4	4
Grade 4 inter. X ? ♂	intermediate	0	6	9
Grade 6 inter. X ? ♂	intermediate	0	13	8
? X ?	white	13	53	80

Bouquet Canyon, California (orange race)

Mono, California (orange or yellow race)

2 F_2 from orange female, Rio Hondo, California (orange race)

These are not all phenotypically identical. In addition to the white color on the wings in place of the normal yellow and orange, these males usually have white legs, palpi, antennae and body. The male from Alstead, N.H. was not of this type. The Pennsylvania specimens were not examined nor do I have a record of their description. The living males from Rio Hondo, California as well as a white female sister had blue-green eyes, bluish blood, bluish larvae and pupae.

History of the white male brood is given in Table 11.

The white female in B1 died soon after emergence from the pupa without expanding its wings. The males lived for three and four days but could not be induced to mate. In none of these did the proboscis parts unite at the end quarter. Their wings did not harden properly after emergence from the pupal case and were quite abnormal. It was intended to intercross orange brothers and sisters of these white individuals (B1 progeny) but fumigation of the greenhouse at this time killed all the material.

It seems clear that the gene giving this white male and female brood is not dominant but recessive and different from that gene discussed as a dominant sex-limited one. It may be surmised that the wild orange female (A) or its mate was heterozygous for the recessive gene. Had the male been homozygous for it, (white) broods B2 and B3 would have shown homozygotes as well as B1. Half the individuals of A progeny (33 ♀ 47 ♂) should have been heterozygous for the recessive gene. One fourth of the broods from these individuals, therefore, should have produced homozygotes. One out of three did; however, considering the lethality of the homozygotes it is possible that B3 might have had homozygotes which did not reach the late pupal stage when they could

Table 11.- Pedigree involving the white male strain. The original female was from Rio Hondo, Calif., Feb. 2, 1941.

	white ♀♀	orange ♀♀	white ♂♂	orange ♂♂
A. Wild orange ♀	--	33	--	47
All crosses from A				
B1. Orange ♀ X orange ♂	1	6	2	19
B2. Orange ♀ X orange ♂	--	26	--	27
B3. Orange ♀ X orange ♂	--	8	--	14
Crosses from B3				
C1. Orange ♀ X orange ♂	--	21	--	18

be distinguished. The cross from B3 progeny shows that some individuals did not carry the recessive gene for white.

Egg Fertility in Various Crosses

Counts on egg fertility have been made in hybrid crosses (yellow race X orange race) and in crosses in which the homozygous dominant white might be expected.

The former were made in order to determine whether or not the interracial segregation of genes has led to any lowering of viability, or to the elimination of any portion of the resulting segregants at this time. The latter were made in order to determine if the expected homozygous lethal dominant would show up in the results by a higher than normal death rate in the egg. The counts were not carried on through the larval stages because of the high normal mortality at this time which could not be controlled.

The classification into fertile versus non-fertile eggs was made by direct observation. Fertile eggs will turn red in about one day at 25-28° C.; non-fertile eggs will remain the newly-laid color of white or yellow for several days before collapsing. The counts were made on the day following time of laying - always 20-30 hours later. This is ample time for the development of all fertile eggs but yet too short a time for larval emergence. All eggs received identical treatment. They were laid on alfalfa leaves at 26-28° C., 80% Relative Humidity and remained at these conditions for one day. Therefore, the effects of normal desiccation should be the same on all eggs.

As a basis for comparison of the various egg fertilities, counts were made of eggs from normal orange and white females. These are shown in Table 1~~5~~2.

The results indicate that two percent or less is the normal frequency of infertile eggs laid. The high infertility (8.6%) of the

Table 12.- The comparative fertility of eggs from wild females. The ratios are given as an indication of whether or not homozygous dominants are expected. The ratio under Westley wild eggs is the ratio of adult females in the population, not that derived from the eggs.

Source of the eggs	Ratio, w♀: or♀	%infertile	N
Westley, Calif.,orange ♀	21: 18	2.6 ± 0.7	502
" white ♀ (2-2)	28: 0	8.6 ± 2.2	151
" white ♀	14: 10	4.4 ± 1.5	180
" wild eggs (random)	74: 26	1.1 ± 0.4	662
Elsah, Illinois, orange ♀	0: 20	2.0 ± 0.5	792
Elsah, Illinois, orange ♀		1.0 ± 1.9	294
Elsah, Illinois, white ♀ (5-5)	18:21	1.3 ± 0.4	761
Round Valley, Calif. white ♀ (1-1)	6: 0	2.2 ± 0.8	361
Total wild females and eggs, orange race		1.9 ± 0.2	3,703
Round Valley, Calif. y♀ X y♂		9.5 ±	42
"		1.7 ±	180
Total wild females, yellow race		3.2 ± 3.2	222

eggs in which homozygotes might be expected (Westley 28:0) is probably not significant. Despite the expected high frequency of homozygotes in the Westley wild eggs (24%), there is no reduction in egg fertility (1.1%). The eggs from Illinois females with no homozygotes expected give the same results as the California material.

Within the yellow-race, egg fertility counts were obtained on two females only. The results are not essentially different from the orange race.

The crosses made to test the genotype of the variant white females (see pedigree, Table 7) yielded the data in Table ~~8~~ 13.

There is no essential difference between the results whether the female is white or orange or whether the female was a homozygote or heterozygote dominant. As the male was a heterozygote, all crosses with white should have produced homozygous dominant eggs, one fourth with heterozygotes and one half with homozygotes.

Results from another pedigree (Table 14) are similar.

The higher infertility of D7 eggs compared with the other white females may be related to the outcrossing since its orange sister has the second highest rate. The difference between the inbred and outbred crosses is significant but the meaning of this difference is unknown.

A lowering of viability might be expected in crosses between the two complexes - orange-race and yellow-race. Though the sterility involved between these is most probably caused primarily by deficient diet, new and unsatisfactory genic recombinations are anticipated which would lower the viability of the subsequent generations. The counts in Table 15 were made ~~for~~ to test~~ing~~ this hypothesis, even though it is expected that mortality will probably occur at later stages as well (that is, larvae and pupae).

These results all show a significantly higher rate of infertility - even as far removed as the backcross. Sisters of the females used in

Table 13.- Fertility in the eggs of different whtie females mated to the same male. See pedigree in table .

Source of the eggs	genotype	% infertile		N
A. white ♀ (2-2)[3]	heterozygote	----		---
B. white ♀ (1-1)	?	0.0		19
C. white ♀ (3-3)	homozygote	0.9	±	907
D. white ♀ (5-3)	~~?~~	2.3	±	44
E. white ♀ (5-5)	heterozygote	0.6		1,194
F. white ♀ (5-5)	heterozygote	1.9		537
G. white ♀ (2-1)	homozygote	2.4		330
H. orange ♀ (6-6)	homozygous rec.	2.4		783
I. orange ♀ (2-1)	?	1.6		386
Total for all females by same male		1.4	± 0.2	4,200

Table 14.- Egg fertility in crosses giving aberrant ratios of white females and also showing a possible difference in inbred versus outbred crosses. The matings are all by a single heterozygous male (pedigree in table).

Source of the eggs	genotype	% infertile	N
D2. white ♀	?	0.6	710
D3. white ♀	?	2.0	494
D5. white ♀	heterozygote	1.9	592
D8. white ♀	?	0.7	451
D9. white ♀	?	0.2	419
Total of the inbred females		0.9 ± 0.2	2,666
D6. orange ♀	homozygous rec.	2.7	866
D7. white ~~orange~~ ♀	" dom.	3.6	837
Total of the outbred females		3.1 ± 0.4	1,703

Table 15.- The frequency of infertility in eggs involving intermediate segregations between the orange and yellow races.

Source of the eggs	genotype	% infertile	N
From Round Valley, Calif.:			
Grade 1♀X Grade 10 ♂	F_1 hybrid	22.4	49
Grade 1 ♀ X Grade 6 ♂	backcross	2.9	345
Grade 4 ♀ X ? ♂	prob. backcross	9.1	629
Grade 6 ♀ X ? ♂	prob. backcross	7.4	108
Grade 6 ♀ X ? ♂	prob. backcross	3.3	423
Total F_1 and backcrosses (Round Valley stock)		11.5 ± 0.9	1154
Sisters of D1-D5, D8-D9 ♀♀ (table) in the following:			
White ♀ X Grade 5 F_1 hybrid ♂	backcross	5.7	697
White ♀ X Grade 6 F_1 hybrid ♂	backcross	10.1	248
Total of these two backcrosses		6.8 ± 0.8	935

the two backcrosses to F_1 hybrids were crossed ~~inter se~~ to brothers with the following comparison: ~~inter se~~ to brothers - 0.9% infertility and backcross - 6.8% infertility.

The F_1 cross with 22.4% infertility in the egg was accompanied by aberrant development as judged by the disturbance in red pigmentation of the egg. The disturbances are such as to cause various portions only of the egg to be pigmented or for the pigment to be lighter in color than normal. Cases of this sort have been noted in the eggs of many females obtained at Mono Lake and Round Valley, California, where the yellow and orange races hybridize. Actual counts show disturbances much as in this cross:

full red eggs (normal)	26
full orange (lightly pigmented)	4
top portion only red	5
lower portion only red	1
tinge of orange only	2
white	11

The yellow female was bred on red clover and the orange male on alfalfa. Therefore, diet should have had no influence on the result.

It is clear that considerable disturbance of development occurs in the inter-racial crosses together with the lowered egg fertility.

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General Summary and Conclusions

1. The genetic difference between the orange and yellow races of Colias chrysotheme is a multiple factor one, as shown by interracial crosses. Hybrid individuals are usually fertile.

2. The two races have a different larval diet ~~necessity~~ even though females of each have been recorded as laying eggs on nearly the same species of food plants.

3. Sterility, sexual apathy, high mortality and phenotypic alterations are the result of interchanging the normal food of the races.

4. The yellow race is able to go into diapause during the middle of the larval stage; the orange race is not able to do so.

5. The ~~food necessity,~~ diapause, and color differences between the races are genetically controlled but are not interexchangeable between the races. However, yellow "orange-race" butterflies do occur rarely in pure orange-race populations.

6. Intermediates between the races are found in the wild. ~~Genetic results indicate that~~ these may be F_1, F_2, F_n or backcross individuals. They are fertile. Copulated pairs of many combinations have been taken in the wild.

7. The white female forms occurring in wild populations of both races are genetically controlled by a dominant allele (confirmation of Gerould, '23). The homozygous dominant is not distinctly separa~~ted~~ble from the heterozygote phenotypically, though there is a suggestion that it might be represented by more extremely white individuals. All three expected genotypes occur in wild populations and have been bred in the laboratory.

8. The homozygous dominants seem to be lethal or semi-lethal

under certain genic environments (modifiers). There is also a possibility that the homozygous recessive may also be at a disadvantage when with a certain modifier ~~environment~~. These modifiers are probably selected by the physical factors of the environment in the wild.

9. The dominant gene for white is readily and easily transferred from one race to the other (confirmation of Gerould, '23).

10. The white males known in wild populations are not genetically controlled by the same dominant factor as the usual white females but by a nearly lethal recessive gene. It also has additional phenotypic effects. White males are found in both races.

11. The normal egg fertility of Colias is approximately 97%-99% fertile. The presence of the homozygous dominant for white females does not affect this figure.

12. There is a lowered egg fertility in the interracial crosses and in the ensuing crosses between intermediates and backcrosses. This is partially accompanied by aberrant egg development.

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Footnotes

[1] Data from Comstock '27, Field '38, Holland '31, Wright '05, Scudder '89, Edwards '68-'93, Clark '32, MS, Gerould MS, Klots '30, Garth '35, Comstock '40, etc.

[2] The point of including this lost or discarded material in this table is to accurately place it in pedigree form. The material was used for egg counts and will be considered on page .

[3] The percentage of fertile eggs from this cross dropped daily from 15% to 100% infertile in gradual degrees within five days. On the eighth day, the female was remated to another male with 39 fertile to 0 infertile eggs. Presumably, she hadn't enough sperm from the first mating.

Hybridization and Seasonal Segregation in Two Races of a Butterfly Occurring in the Same Place

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The yellow and orange butterfly, Colias chrysotheme, exists in the form of two complexes known as the orange-race and the yellow-race (Hovanitz, '43a, '43b). These races have different geographical distributions but overlap over a tremendous territory from the Sierra-Cascade divide in western North America to the Atlantic ocean in the east and from southern Canada in the north through Mexico in the south (Hovanitz '43c). Each race usually occupies a different ecologic niche so that nearly pure populations of each may be found in this area as well as outside the zone of overlap. In certain places, however, the same ecologic niche is partly occupied by both races, resulting in considerable hybridization between them.

Two places where the races occupy the same niche for the most part where analyzed in 1941-43 in order to study the behavior of each in relation to its environment and to get an indication of the extent of hybridization between them. These were at Mono Lake Valley, Mono County, California and at Round Valley, (near Bishop), Inyo County, California. The positions of these localities are indicated on a map in another paper (Hovanitz, '43d); they are just east of the Sierra Nevada in the western Great Basin.

The Seasonal Distribution of Adults

Orange butterflies are present throughout the entire warm season of the year at both Round Valley and Mono Lake. It is easier, however, to get a good sample in midsummer as compared with early spring or autumn. The abundance of orange adults apparently is at a minimum at each end of the growing season and at a maximum in midsummer.

The yellow butterflies at Mono Lake are more irregular in seasonal distribution than the orange (fig. 1). The 1941 samples (Table 1) show a high frequency of yellow to orange in May, and then a complete drop to none present at all in June. A rise to a second maximum in late July is apparent with a gradual drop again to none at all in September. Early in October there is a third maximum. This suggests three distinct broods per year at Mono Lake with an elapsed egg, larval and pupal development time of two months between each. This time compares with a development rate of three to four weeks at a constant laboratory temperature of 25° C. Mono Lake has a rather low air temperature, especially at night; in the day time, the direct radiation from the sun is the primary source of heat.

The 1942 samples at Mono Lake show much the same seasonal distribution. The first adult flight was apparently not observed; it is probably very short in duration. The 1942 samples were obtained at monthly intervals rather than semi-monthly as in 1941; therefore, the chance of missing a short adult flight is increased. The second and third broods of 1942 are indicated a few weeks earlier than in the preceding year. 1942 was a warmer year for Mono Lake than was 1941. An earlier spring start in larval development with a consequent shift forward in the successive broods would thus be expected.

The two 1940 samples at Mono Lake show no yellow butterflies present at all. Compared with 1942, it would appear that they were obtained in a yellow interbrood period (fig. 1).

The frequency at Round Valley does not follow this sequence of events (fig. 1). Neither the 1941 nor the 1942 samples show any correlation with those of Mono Lake (Table 1). This shows the complete lack of intermixing between the two places though they are only fifty miles apart. The 1941 Round Valley curve is high in late June (60% yellow) and drops to a low in late July (25% yellow). A rise occurs

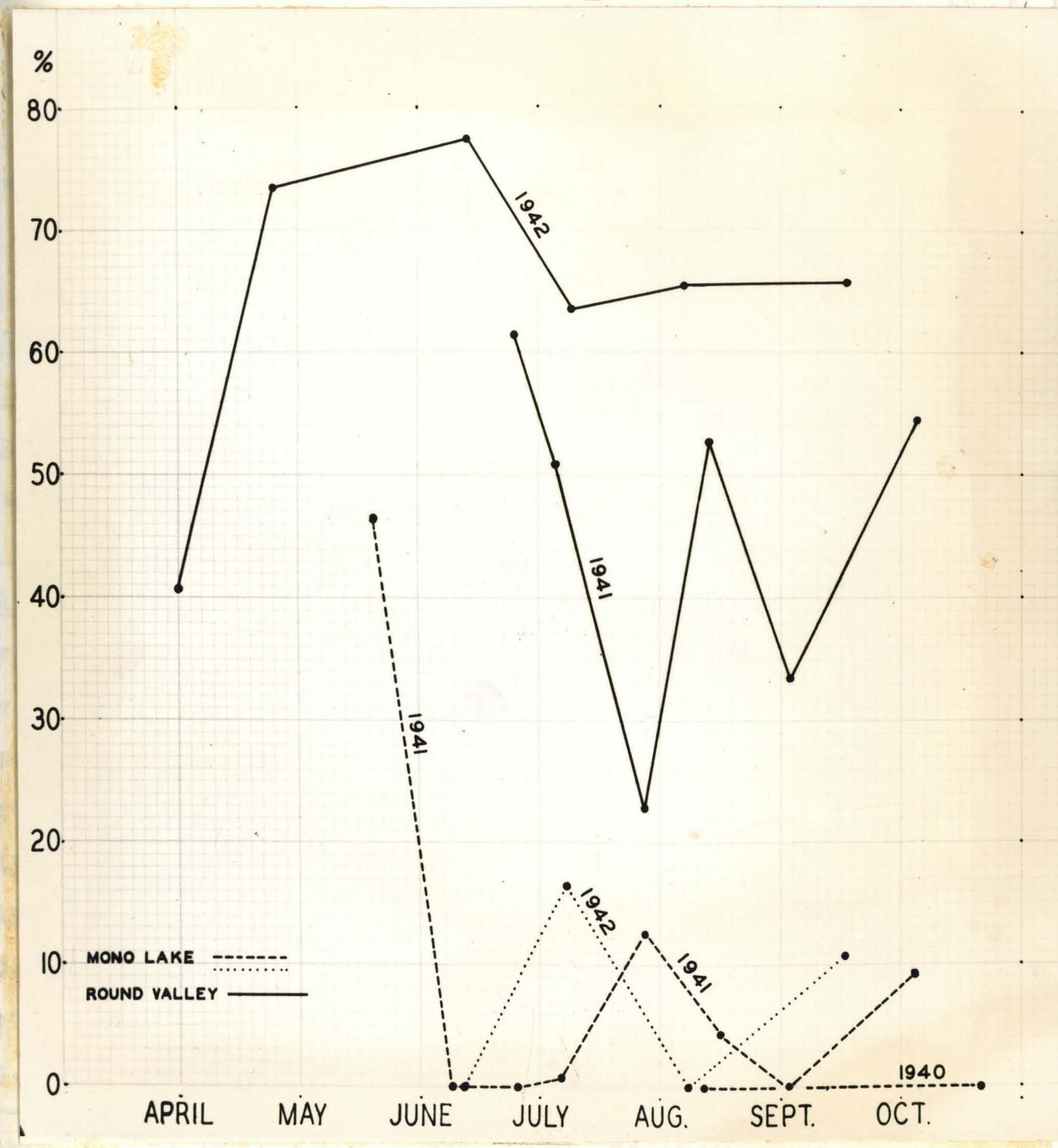

Fig. .-- Frequency of yellow to orange butterflies at Mono Lake and Round Valley, California throughout the season. Note the complete absence of yellow at certain times at Mono Lake.

Table 1.- The frequency of the yellow-race as compared ~~the~~ with the orange-race butterflies at Mono Lake and Round Valley, Calif.

	ROUND VALLEY		MONO LAKE	
Date	% yellow	N	% yellow	N
1940	---			
Aug. 11	---	---	0.0	105
Oct. 20	---	---	0.0	46
1941	50.50 ± 2.89	299	5.84 ± 0.63	1,387
May 4	many	many	---	---
May 19	---	---	46.27	75
June 8	---	---	0.0	91
June 24	61.19	134	0.0	70
July 5	50.82	61	0.89	678
July 26	22.92	48	12.66	237
Aug. 15	52.63	19	4.21	95
Sept. 2	33.33	15	0.0	20
Oct. 4	54.55	22	9.01	121
1942	65.96 ± 1.50	987	10.68 ± 0.88	1,236
April 1	40.82	49	---	---
April 25	74.55	110	---	---
June 12	77.14	140	0.0	many
July 7,8	63.70	540	16.16	396
Aug. 6,7	65.22	69	0.0	434
Sept. 16	65.82	79	10.67	406
1940-41-42	62.36 ± 1.35	1,286	7.68 ± 0.51	2,774

in mid-August (53%) with subsequent drop again the first of September (34%) to a last rise in early October (55%). If these fluctuations represent successive broods not completely separated one from the other, then there are many more generations present per year at Round Valley than at Mono Lake. This would be expected considering the warmer climate at the former place (Round Valley is at an elevation of 4500 feet and Mono Lake at 6500 feet). Latitudinal differences in brood number per year parallel these altitudinal ones. There are three generations per year near Washington, D. C., (and Mono Lake), two generations near Hanover, N. H. (and central British Columbia) and one generation in Alaska and Yukon Territory. At Round Valley there are probably four or five.

The 194~~5~~7 samples are more extensive at Round Valley than are those of 1941 (Table 1). There is a low of yellow (40%) in early spring, rising to a high within the month of 75% and later 79%, with a rather constant frequency of 65% yellow the remainder of the year. This curve shows little evidence of a series of broods or generations during the year. At the rather high temperatures prevailing in the valley during the summer (around 40° C days and fluctuations but not very cool nights), the succession of generations would be at about one month intervals. The samples were made at this interval of time, so it is quite possible that the two were in phase. Were this the case, the results would show a rather constant seasonal frequency. On the other hand, it is possible that the variations in development rate between individuals owing to micro-temperature differences in the locality have completely eliminated the inter-brood population minima. This has been shown to be partially true for the second and third broods in the vicinity of Washington, D. C. as well as for New York state. In these places, only the break between broods one and two ~~are~~ is clearly defined by the absence of adults.

A higher frequency of yellow at Round Valley than at Mono Lake, at all times, is apparent (fig. 1). Several factors combine to create this difference: (1) more larval food is present at Round Valley (Trifolium), (2) Round Valley is farther ecologically from the source of the migrant orange-race individuals (San Joaquin Valley), for these are more likely to stop in the mountain meadows than to procede through the desert to Round Valley. The frequency of yellow is given as compared with orange. When the orange frequency goes down, the yellow will appear to rise in the curve. (3) The longer and warmer growing season at Round Valley gives more time for the resident population size to be built up. This has been shown elsewhere by the increased numbers of individuals in the second and third broods at Washington, D. C. and New York as compared with the first spring brood.

A higher frequency at Round Valley of yellows in the 1942 over the 1941 samples is also indicated. The latter samples were obtained in a mixed alfalfa-red clover field at the periphery of the large meadow which constitutes the primary ecologic niche for the yellow-race. The 1942 samples were made at a different field one mile from the latter (containing alfalfa, red-clover, white clover and native perennial clovers) in the center of the meadow. This field would be in the midst of the population for the yellow race whereas the former field is on the periphery. For the migratory orange-race (Hovanitz, '43d), no part of the meadow would constitute a population center. The higher frequency of yellow in 1942, therefore, can be accounted for by this change in position of the place sampled.

Hybridization Between the Races

Genetic data on crosses involving the races and on progency from wild intermediates between the races indicate that crossing is easily possible and occurs frequently (Hovanitz, '43b). The indications also are that there is no genetic sterility between the races. The

F_1 is an exact intermediate of a light orange color; F_2 and backcrosses give the range of intermediates expected on a multiple factor distribution of genes.

The range of colors from the parental types through the intermediates is given in a range from yellow to orange of 1 to 10. From genetic results, it is known that grades 1 and 2 are pure parental types, breeding true for the yellow race. In the pure populations of orange race, there is a range of yellow to orange from 1 to 10 but from about 1 to 7 or 8 these are exceedingly rare (Hovanitz, '43e). Therefore, grades 8-10 in the males and 7-10 in the females are considered as "parental types" for the Round Valley population. It is understood that grades 7 or 8 may be intermediates or that some lower grades may be parental types but that these will be insignificantly small.

On the basis of grades 3 through 7 in the males and 3 through 6 in the females, the frequency of intermediates in the Round Valley populations have been calculated (Table 2). It is seen that there is but little seasonal change in the abundance of intermediates (fig. 2). A high of 30% in August, 1941 is possibly a result of the small sample size. An average of about 10% intermediates is usual.

Range of Wild Intermediates

The statistical consequences of continued interbreeding between the orange and yellow races should be a single race combining the characteristics of each parental type. But the two races have maintained their primary discreteness after more than 70 years of such interbreeding, and probably many centuries (Hovanitz, '43b, '43c). Were the interbreeding only of very recent origin, the hybrid range would show a very high frequency of F_1 intermediates (Grades 5 or 6) and a lower range of F_2, F_3 and backcross intermediates (Grades 3-4, 7-8). The data on wild individuals (fig. 3) do not show this higher

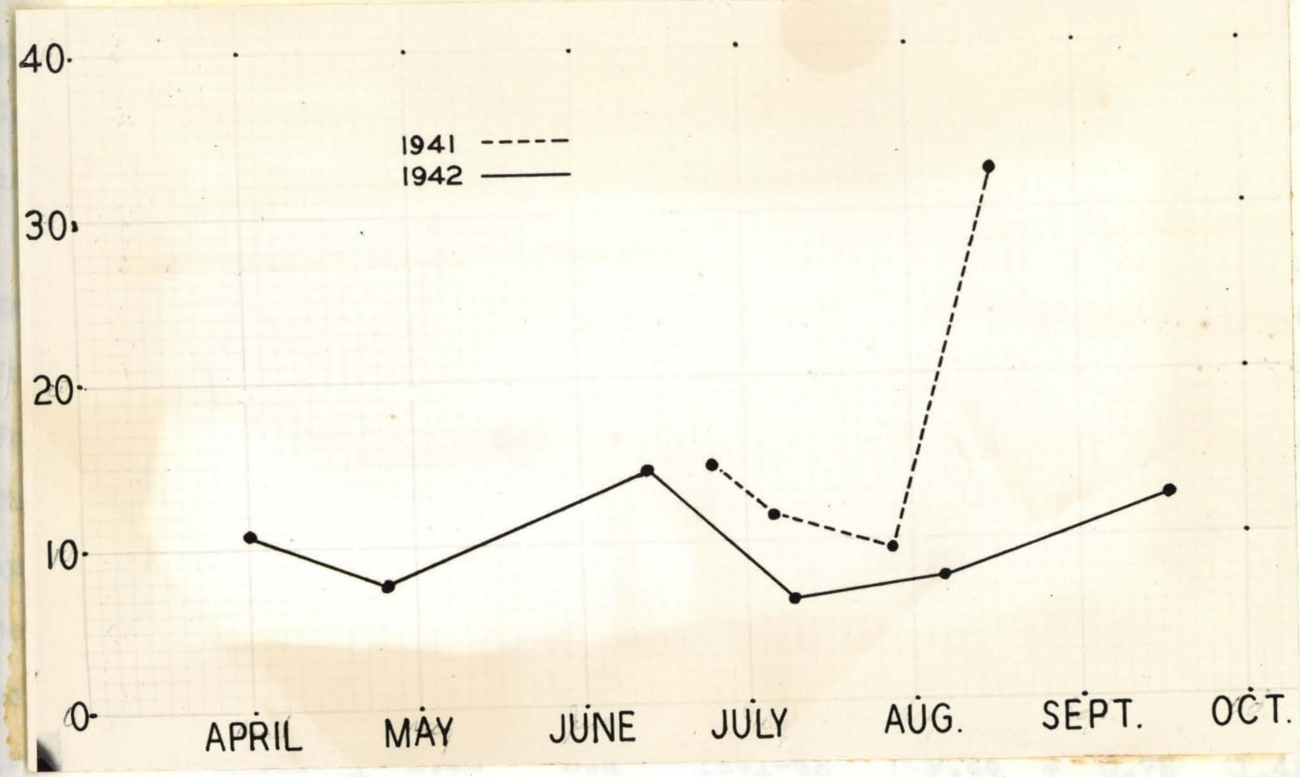

Fig. 2 .-- Frequency of intermediates between yellow and orange in the population at Round Valley, California 1941-42.

Table 2.- The frequency of intermediates in the mixed population of orange and yellow races of Colias chrysotheme at Round Valley, California. (The total given in Table 1 does not include intermediates; hence it is smaller than that given here).

	1941			1942	
Date	% intermediates	N	Date	% intermediates	N
May 4	---	many	April 1	10.91	55
June 24	14.65	157	April 25	7.56	119
July 5	11.59	69	June 12	14.15	163
July 26	9.43	53	July 7,8	6.08	575
Aug. 15	32.14	28	Aug. 6,7	6.76	74
Sept. 2	0.0	15	Sept. 16	12.22	90
Oct. 4	0.0	22	1942	8.27 ± 0.84	1,076
1941	13.08 ± 1.82	344	1941-42	9.44 ± 0.78	1,420

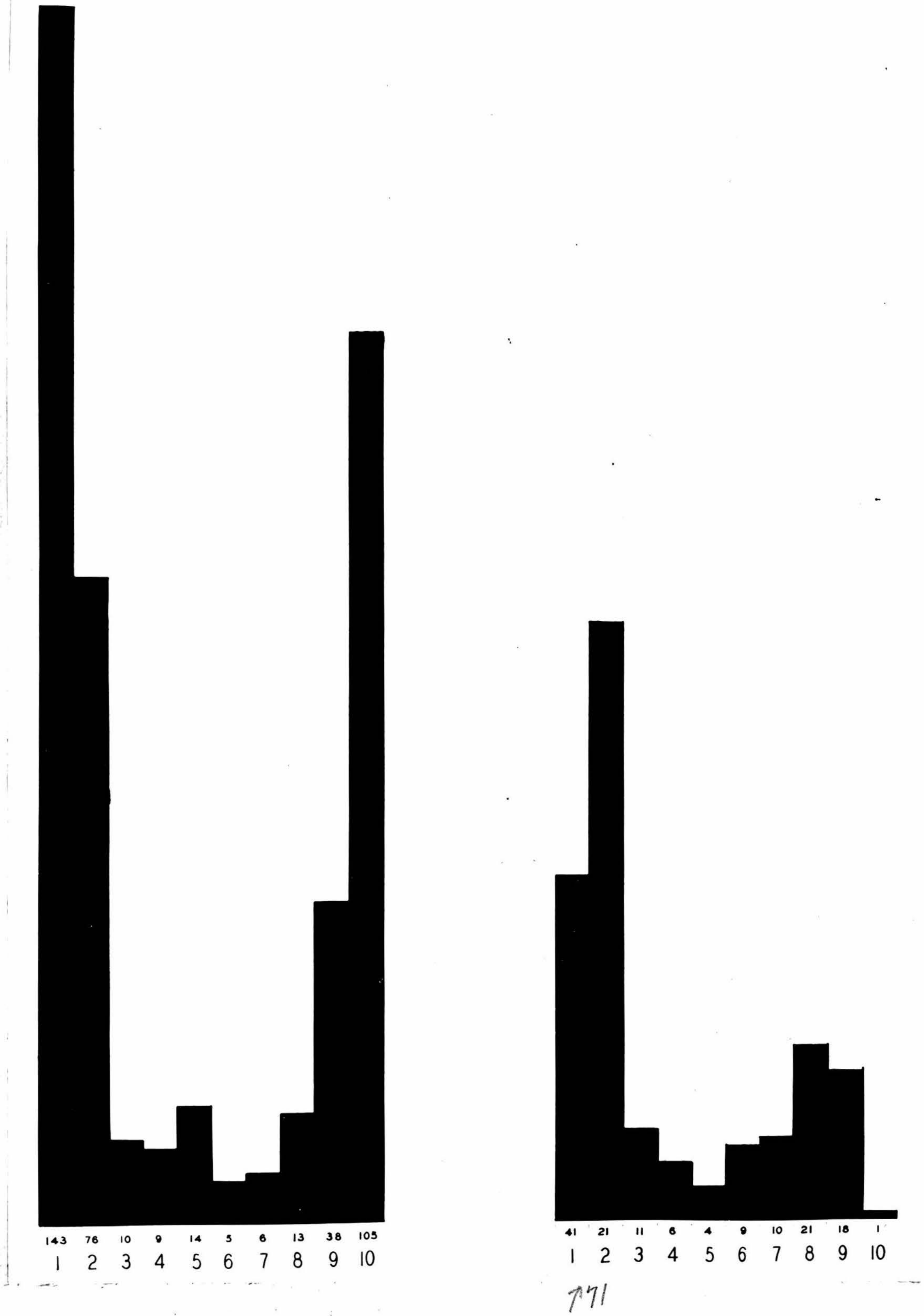

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Fig. 3 .-- Range of variation from yellow to orange in 10 grades (see text) of wild individuals taken at Round Valley, California 1941-1942. ♂ on left, ♀ on right.

frequency of F_1 to any great extent. The female curve may be masked by the normally low orange female grades. The male range shows a somewhat higher frequency of Grade 5 than the other intermediates. The lack of the F_1 intermediates compared with F_n intermediates may be due to many factors of which a general lower viability seems to be the most likely (Hovanitz, '43b).

Discussion

The data on the existence of the two races of Colias living in the same place suggest how ecologic and physiological differences can be maintained in units which may be called species. The races are not here called species, for some genes are easily and often interexchanged (Hovanitz, '43b). However, other genes are not effectively segregated in this way. This suggests that the significant gene complex characterizing each race and giving it individuality is not broken down in hybrid crosses.

Since the color difference separating the races is a multiple factor one and these factors are segregated independently of the basic complex, it might still be expected that a complete intermediate population would be produced, separated only by the non-visible basic complex. The reason for this lack of complete blending of characters probably lies in a combination of the following conditions:

(a) Sexual selection (Hovanitz, '43b) may prevent sufficient intercrossing to be effective.

(b) Eggs genetically determined to be yellow-complex laid on alfalfa will later result in sterile adults or the subsequent larva may die; also the reciprocal on red clover (Hovanitz, '43b).

(c) The intermediates of all types are probably less viable than the parental types and many of them will be sterile on the food plant upon which they feed (Hovanitz, '43b).

(d) The diapause associated with the one complex (Hovanitz, '43b) tends to keep the races ecologically separated.

(e) The supplementary color genes of each normal type probably act better in unison with the basic complex than any intermediate segregation of genes.

(f) The different ecological niche occupied by the food plants necessary for each complex aids in preventing hybridization (Hovanitz, '43c).

Summary

1. Two populations where the races of Colias chrysotheme occur together are described.

2. The yellow-race has definite broods during the season. The orange race in this place apparently does not.

3. The yellow-race has more seasonal generations when a population is at a lower elevation than at a higher elevation. This compares with latitudinal differences of the same type.

4. The two populations are separated by a territory of 50 miles, but show no correlation in seasonal generations.

5. The yellow-race generations at the higher elevation are separated by interbrood periods with no adults. At the lower elevation, the generations merge one into the other.

6. Hybrid intermediates are present at one locality rather constantly at a frequency of about 10%.

7. The range of intermediates is not trimodal as one might expect on the basis of multiple genes but a U-shaped curve. This is probably due to a low viability of the F_1.

8. Several reasons are given to account for the lack of complete blending between the races after years of hybridization.

Acknowledgements

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The Ecological Significance of the Geographical Distributions of the Colias Chrysotheme Races and Forms in North America

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In North America, two major races of Colias chrysotheme can be distinguished. These races are not differentiated by morphological characters, but primarily by physiological ones (Hovanitz, '42, '43a). Correlated with these physiological traits, there is the presence of orange pigment on the upper surface of the fore wings in the orange-race, and the absence of this pigment in that place in the yellow-race. Very occasionally "spots" or genic recombinations occur within the orange-race which will prevent the appearance of orange pigment in the wings. Such individuals are exceedingly rare (Hovanitz, '43b). This color difference of the fore wing has been shown by genetic tests (Hovanitz, '43c) to be of significant accuracy in the segregation of specimens into the major 'orange' and 'yellow' races. It is by means of this color difference that the segregation of museum specimens was made, the data of which are considered in this paper.

Within the wild populations of each of these physiological races, a dominant gene occurs in varying frequencies. This gene controls the substitution of a white-colored pigment for the normally predominant yellow or orange pigment upon the surface of the wings in the female sex. The white females of the two major races are phenotypically indistinguishable in long established populations of the two races in the same area. Consequently, no segregation has been made between white females of the races in the museum material.

According to the zoogeographic 'rule' suggested in a previous paper (Hovanitz, '41), the pterine pigments of the Pierid butterflies

(Group III) should follow a definite distributional sequence with respect to correlations with the environment. The orange-pigmented varieties should be found in warmer locations as compared with the yellow or white varieties. The yellow-pigmented varieties should be found in warmer locations as compared with the white varieties. "Rules" such as these are found to be true in a general way when otherwise similar animals are being considered. That is, when a yellow physiological race is compared with an orange race, the yellow one should be found in the colder or the more northern locations. This is found to be true in the case of <u>Colias chrysotheme</u>.

However, the white-female form is a single genetic mutant occurring in each race. The comparison is therefore made with the normal yellow or orange variety segregating with it in the same population. When this comparison is made, it is found to be true that the white forms are more abundant in the northern or colder areas than the normal orange or yellow forms.

The ecological reasons for the different geographical distributions of these color forms are considered in this paper.

<u>The Past and Present Distribution of the Orange Race</u>

The map (Fig. 1) illustrates the geographical distribution of the orange form. It is noted that the area includes all the territory of North America, as far north as southern Canada, and as far south as the tropical life-zones of the Gulf coast, and Central America as far as southern Mexico. From observers in the field in specific places, and from notes in the literature, it is known that the butterfly reinvades the northern part of its range anew from the south yearly (Bowman, '42). Some times a resident population remains to the next season, in other years it does not. Many of the far-northern localities shown on the map may be considered to be captures of migrant individuals or also adults grown during the one season in the locality

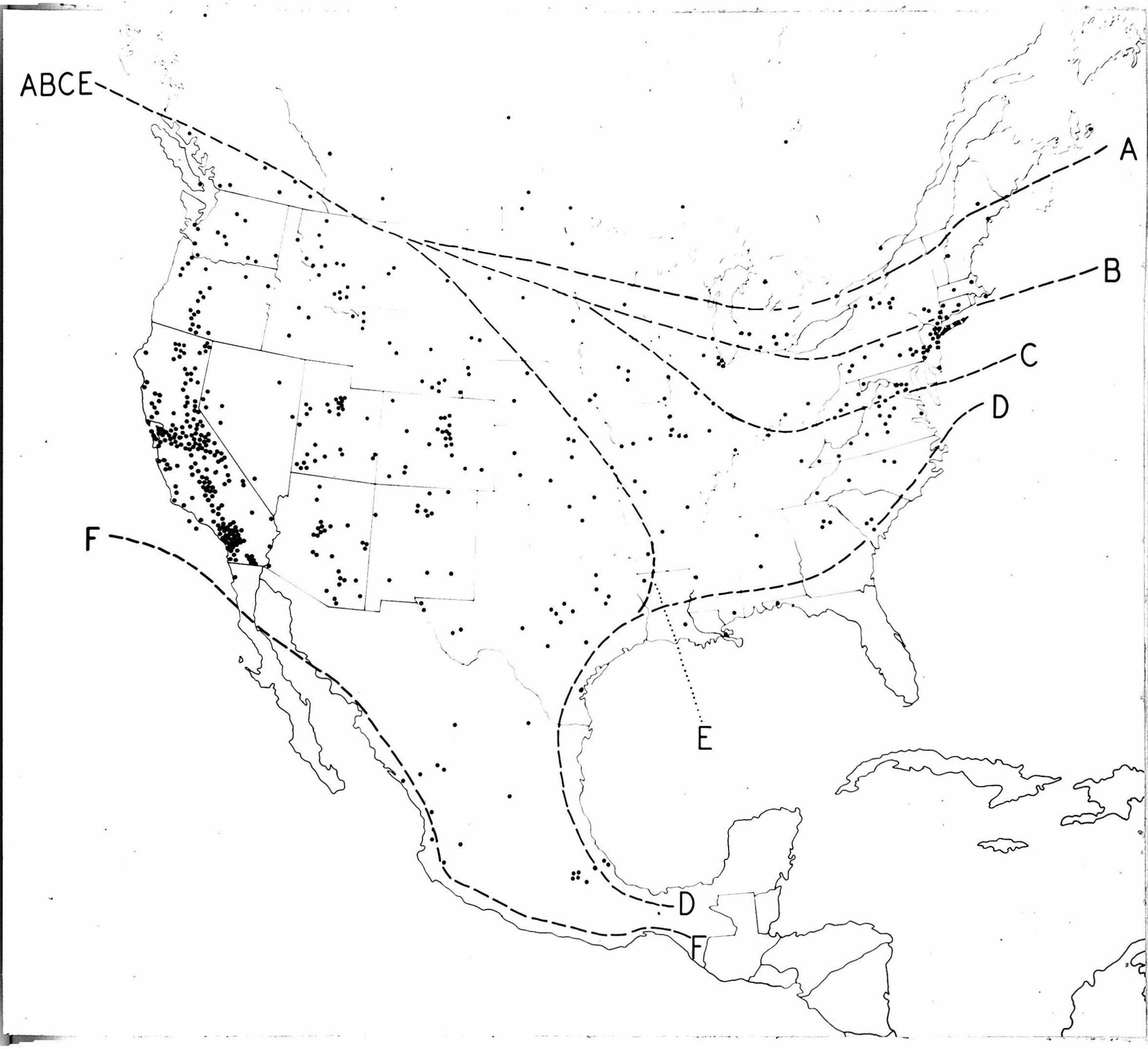

Fig. 11.- The geographical distribution of the orange race of *Colias chrysotheme* in North America (=*eurytheme*). The dashed lines enclose the approximate breeding limits of the race. The line E-E is the eastern-most distribution prior to about 1800. C-C = extension of range from about 1880 to about 1925. B-B = extension to about 1929. A-A = extension to about 1943 or present northern breeding limits.

from eggs laid by migrants. One definite reason for the inability of this form to exist in the far north is its lack of winter diapause, though other factors may also be involved (Hovanitz, '42,).

The distributional area is limited on the south by another climatic barrier. Breeding populations are rare and scarcely known in the hot, humid Gulf coast strip of Mexico, on the west coast of Mexico in the humid areas and likewise south into Central America. Dr. Tarsicio Escalante has been kind enough to describe the general distribution of this butterfly in Mexico from which I quote: "Occurs at 800 to 1000 meters above sea level to 2800 or more. Most frequent in the Central States, such as Chihuahua, Coahuila, Durango, Zacatecas, Agua Calientes, Guanajuato, San Luis Potosi, Queretaro, Puebla, Tlaxcala and Mexico. Occurs also in the states of Tamaulipas and Vera Cruz but far from the sea and under the same conditions in Sonora, Sinaloa, Jalisco, Nayarit, Colima and Michoacan. Does not occur between the sea and the Sierra Madre nor in the southern calid states of Chiapas, Tabasco, Campeche, Yucatan and Oaxaca. Is very rare in Morelos and Guerrero." This information, in addition to that given on the map shows that the butterfly exists primarily on the Mexican plateau at moderate to high elevations and is absent under the humid tropical conditions.

The present distribution of the butterfly in the United States has been much increased over what it was but a few decades ago. The butterfly was not present east of a line drawn from north to south along the eastern edge of the Great Plains prior to a few decades before the turn of the twentieth century[1]. The exact times at which the butterfly appeared in breeding proportions in the southern Appalachians is not very well known. At any rate, from the line drawn as above and as shown as the western-most dotted line on the map (Fig. 1) the migration took place yearly in a north and east direction. Sometimes years of migrations were necessary in order to establish a population in any given place. Forbes (personal communication) notes at Ithaca, New York: "Single scattered specimens for many years over the whole area, then will suddenly occupy a spot and become abundant within a few years, the date of this change varying widely in closely neighboring places. Ithaca had single specimens in 1927, then abundant over a few alfalfa fields in 1928; then common everywhere in 1929 and later, but most abundant over alfalfa fields." These observations are corroborated by several other witnesses. Similar cases are known for other parts of the northeast. Dr. G.W. Rawson of Detroit, Mich. had never seen the orange race in that vicinity about 1923, whereas it was quite common at that time at points from Kentucky south. The orange became common about 1930 and has actually established itself in breeding populations until it is now apparently commoner than the yellow form. It is noted that the occurrence annually is erratic, suggesting a difficulty of this form in overwintering in the region.

There are data on orange specimens in Buffalo, N.Y. as early as 1921, Brooklyn, 1918, Staten Island, N.Y., 1921, and an old specimen taken before 1900 is labelled New York. The earliest records for some New England states are 1932 in Connecticut, 1929 in Massachusetts, 1916 for New Brunswick, etc., suggesting an erratic occurrence (see Scudder's records below). An Ottawa, Ontario record is present for 1900, Pennsylvania is recorded for 1910, Tennessee for 1895, Virginia for 1916, West Virginia for around 1878. Overwintering populations certainly exist throughout all the east now.

It is not certain whether Illinois has been a breeding place for these butterflies prior to white man's entry or not. Occasional specimens, at any rate, must have been very common. Some old dates are: Evanston, 1891, 1900, 1905-6, Chicato, 1907, Quincy, 1900. Belvidere, 1897, 1900, 1892, Peoria, 1872-5. This old material in fair abundance suggests proximity at least to breeding populations. The rarity in adjacent Michigan suggests that Illinois itself was not a strong source of migrant material. Old material of the middle west collected by Mead, Edwards, etc., but without specific localities, suggests the presence of this butterfly in this region prior to the coming of man's alterations. The earliest record in present museum material from Kansas is 1909 but they were certainly here earlier. From Columbia, Missouri, there are some "before 1900" and one 1897. From the Rocky Mountains west there is no doubt of its presence long before European culture arrived in North America; it was originally made known from San Francisco in 1833.

Scudder ('89) writes of the distribution at that time: "most abundant in Mississippi Valley and west between isotherms 40° and 70°. Occurs sparingly east of Mississippi Valley and entirely absent at the Alleganies except for a few places as below[2]. Prevails over <u>philodice</u> in the Mississippi Valley. East of Alleganies known only from Newcastle, Del., Maryland, Georgia, in New England known from Norwich, Conn., Wollaston, Belmont, Mass., Montpelier, Vt., Mt. Desert, Me. Apparently migrates down the St. Lawrence Valley as it is known from: Michigan, Bruce Mines, London, Ont. (occasionally), St. Catherines, Ont. (rare), Montreal, P.Q., Quebec, 20 mi. below Quebec, Missiquoi Co., P.Q., Albany, N.Y."

According to Scudder ('89) prior to 1889, it was taken as far north as Vancouver Island, in the west, Dakota, Milk River, Montana, Lake of the Woods, Nepigon north of Lake Superior, Moose, Albany River (on the Hudson Bay) and Athabasca region. In the southeast, it was known at this early date from southern Texas, New Orleans, La., Oxford, Miss., and "Alabama" but was rare in the latter three places. Dury states that it was rare in southern Ohio.

Clark (MS) believes that the orange form moved into the state of Virginia and the vicinity of Washington, D.C. from the south or southwest. He cites an observer (Smyth) who never saw this form in Blacksburg, Montgomery County up to 1925, although he was a good observer. An observer (Rawson) never saw it till 1925. Another observer (Jones) in 1894 saw no orange north of Salisbury, Rowan County, North Carolina, on a trip through the region; the yellow form was first observed at that time in Danville, Virginia. Yellow forms were common at Danville Beach in 1906 but no orange were there. This same observer first noted the orange form on the Delaware peninsula in 1920 at Ocean City, Maryland. In 1923, yellow were common around Wilmington, Delaware, but no orange until south of Berlin, Worcester County, Maryland. At Wachapreagul, Accomac County, Virginia, there were only orange. In 1925, orange was found in Dismal Swamp. In 1925, orange was more abundant than yellow, at least as far north as Lexington, Rockbridge County; north, yellow supposedly was more common (A.B.J. Clark, from Clark, MS.).

Clark finds that the first District of Columbia record for the orange form was one by Lugger in 1896. Other single records are 1904, 1910, and 1923. In 1925 and 1926, it had become fairly numerous in the vicinity. In 1927, it was exceedingly abundant. In higher localities as at Silver Springs and Somerset, Maryland, in 1927, there were about one half or equal numbers of yellow and orange forms. These early records were nearly all from August to the end of the season. In 1928, specimens were noted in June; in 1929, on May 19, but in this case disappeared again for a few weeks until June, presumably during an interbrood period. In 1930, it was noted in April and later was more abundant than the yellow form.

It was quite common in 1889 at Columbia, S.C. and was bred on clover. Yellow forms were absent at that time at Charleston and Columbia, S.C. Brimley and Sherman state that the orange were abundant over the state of North Carolina from at least 1900 onward. W.H. Edwards ('68-'93) did not find the orange form in the Kanawha Valley of West Virginia (Coalburg) in the 1880's. Clark (MS) has found it common in 1929.

The long dashed lines shown on the map enclose roughly the area within which breeding populations of more than a single season exist. Areas outside these lines are considered as of insignificant importance in the breeding population. The three dotted lines show the northeastward extension of range since man disturbed the ecological conditions in the east.

The best reason the author can give for the present extension of range of the orange form into the east and northeastern parts of the United States is the opening up of new breeding places by removal of the original forest cover and replacement by plants which can be used as larval food by the butterfly. In the wild this significant replacement has been by sun-loving legumes such as Astragalus and Vicia, which can be used as food by the orange form. Under cultivation, the now extensive growing of alfalfa has provided a new source of food. This taking-over of an introduced plant by a native species has greatly enlarged the total area occupied by the species, as well as the total numbers in existence. Under native conditions, the butterfly could hardly ever have been very numerous owing to the scattering and scarcity of food. The habits of the insect in such areas are those of a migratory one. With the taking on of the role of a parasite on alfalfa and therefore as an agricultural pest of man, the butterfly has become so common as to destroy fields of this crop.

The map (Fig. 2) shows the present distribution of alfalfa as taken from the Agricultural Yearbook, 1929. The distribution of alfalfa acreage as shown on this map corresponds almost exactly with the

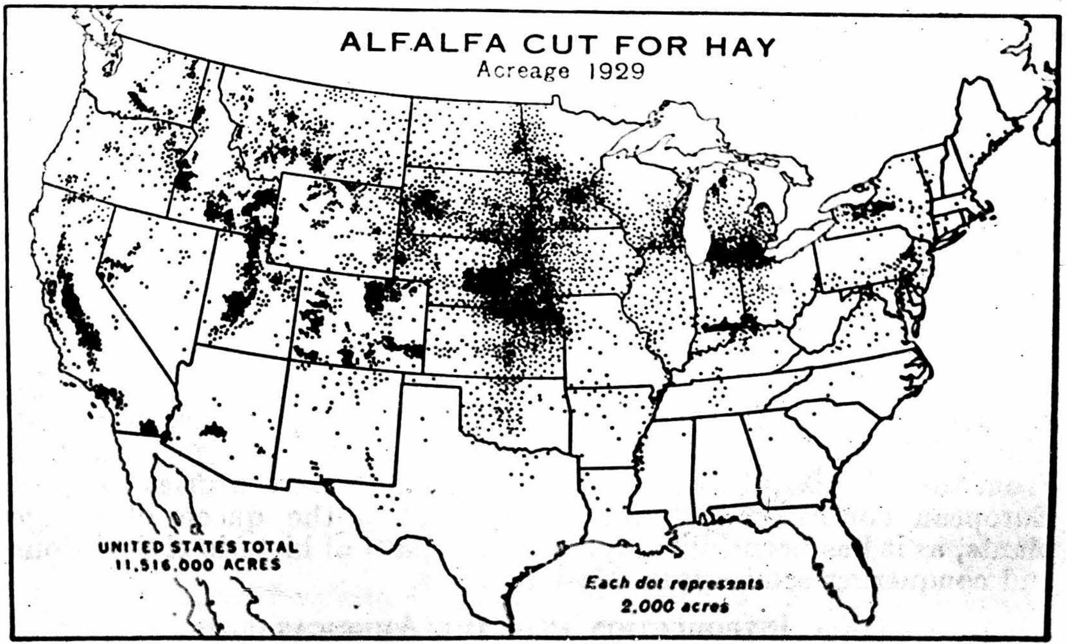

***Figure 1.*—Distribution of alfalfa production in the United States in 1929. Approximately 11,500,000 acres were cut for hay in that year.**

Fig. 2.- Approximate acreage of alfalfa in the United States in 1929. Compare with figure 2. Note the original distribution of the orange race and compare with the present distributions of that race as related to the growing of alfalfa.

present distribution of the alfalfa butterfly (the orange form). In the areas in which the author has personal field experience (California, Nevada, and Arizona) the butterfly is most abundant in direct relation to the abundance with which larval food is available. As alfalfa is the food which at present is most suitable and usable by this butterfly, the abundance of individuals can be directly correlated with the abundance of the acreage of alfalfa grown. Physical environmental conditions are apt to be effective in reducing the population numbers, however. Thus, California valleys probably have a higher number of butterfly individuals per acre of alfalfa than the midwest or the east. In the north and east, cold winters serve to reduce the population to lower numbers; in the south and east, hot and humid summers serve to reduce the population by killing the insects or sterilizing them.

Alfalfa was first grown in North America in effective quantity by early settlers of California about 1850[3]. The later irrigated lands of the California valleys provided perfect conditions for its growth. From California it was taken to the Great Salt Lake Valley, where the Mormons found it satisfactory on the irrigated desert lands. In 1868, the first fields were established in Kansas. Likewise, about the same time in Colorado and Nebraska. The crop was very abundant in 1890 in Kansas and but little less so in Nebraska. At least by 1900 it was east of the Missouri River on the alluvial soils of Iowa and Nebraska. A little later it was established in Illinois and Ohio (Tysdal and Westover, '37). According to Bailey ('07), alfalfa was first established in 1867 in New York state in Onondaga County and was very common by 1894. According to Forbes (personal communication), it was not until 1928 that the alfalfa butterfly really became well established in upper New York. Bailey ('07) states that in 1891 there were 34,381 acres of alfalfa in Kansas; by 1906 this had increased to 614,813 acres. United States acreage in 1899 was 2,094,011 acres (Bailey, '07); in 1929 it was 11,516,000 acres (Tysdal and Westover, '37). The orange alfalfa butterfly has had, therefore, remarkable encouragement for multiplication in numbers.

When man removed the forest cover over the eastern states as far west as the Mississippi, he removed one of the barriers to the distribution of this butterfly eastward. The food plants upon which the larvae feed generally live in full sunlight, as before mentioned. The opening of more such terrain has opened the possibilities for a greater population size, irrespective of the greater possibilities as shown above by alfalfa fields. Despite the dearth of alfalfa in the southeast (see Fig. 2), the orange alfalfa butterfly is fairly common outside the immediate Gulf and Atlantic strips. Presumably this is due to the opening of the forest cover and increased abundance of other food plants. Dr. Tarsicio Escalante (personal communication) notes that in Mexico, the butterfly distribution is apparently correlated with that of alfalfa and is increasing as the growing of alfalfa is increased. The total population size has, without doubt, been greatly increased by the introduction of this plant everywhere.

Summary: The orange race originally occupied a range from southern Canada to southern Mexico and from the Pacific ocean to the central portion of the Mississippi Valley. With the opening of new ecological conditions in the east, the range has been increased in that direction as far as the Atlantic ocean.

The Distribution of the Yellow-race

The map (Fig. 3) shows that the distributional area of the yellow-race is more extensive than that of the orange. The land area enclosed within the dashed lines represents the area potentially

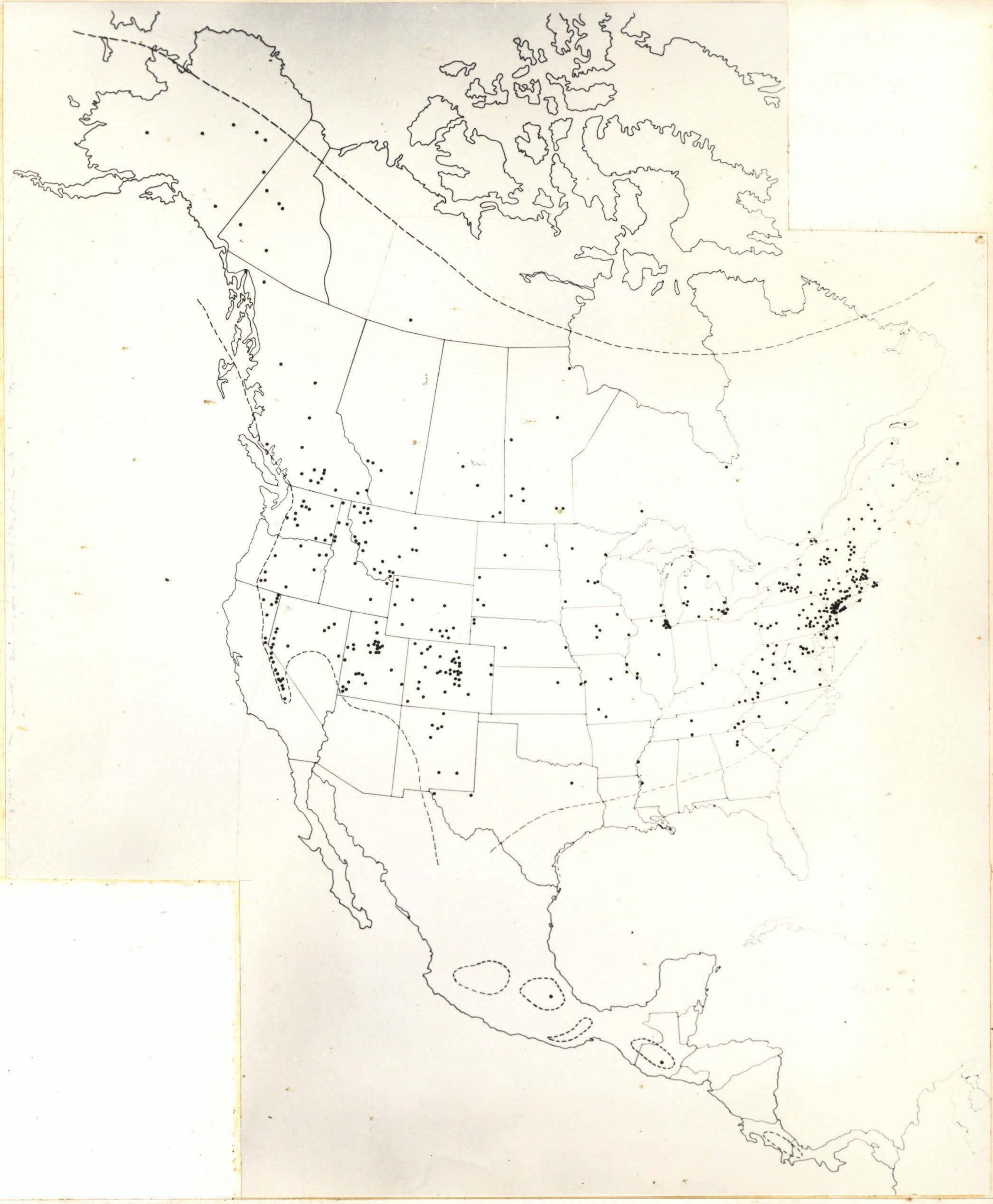

Fig. ~~2~~3.- The geographical distribution of the yellow race of *Colias chrysotheme* in North America. The dashed lines enclose the approximate breeding limits of the race. Note that in Mexico and Costa Rica certain areas are noted as potentially breeding areas. Each dot represents a known locality listed in the appendix but not the abundance of the race.

expected to provide living conditions for the insect. The ecologic niche for this butterfly is narrower than for the orange within this area. Apparently wet places where clover can grow throughout the season are essential. Therefore, in the dryer parts of the continent it is limited to wet meadows where clover grows throughout the year, dependent upon water run-off from the nearby high mountains.

Along the river courses of Alaska, Yukon Territory, and the southern part of the Northwest Territories, this form is found, south of the tundra. Collections of butterflies north of the line of tundra (Baffin Island, Southhampton Island, Boothia Peninsula, Greenland, etc.) failed to show this Colias present, though other species of Colias are quite common (nastes, boothi, hecla, etc.). This insect is apparently widely distributed throughout the whole of southern Canada.

In the United States, the yellow race is absent in two significant regions: (1) the Pacific Coast strip west of the Cascade-Sierra Nevada divide, southern Nevada, and Arizona and (2) the Gulf Coast, Florida and the southeastern lowlands. The absence on the Pacific Coast is believed due possibly to the dry summers prevailing in this area making extensive patches of wet meadows for clover propagation rare. Or possibly it is due to the warm winters prevalent here, or to the fact that where in the north summer precipitation is present, the climate is cool and overcast much of the time. On the Gulf Coast, apparently the combination of too warm a temperature and humid an atmosphere of this subtropical climate is the limiting factor.

In Mexico and Guatemala, the butterfly is limited to very high mountain meadows far above the Mexican plateau and above that zone occupied by the orange form. Potential areas of occupation are encircled with a dashed line in Mexico and Costa Rica (Fig. 3).

Throughout the eastern parts of its range, this butterfly (yellow race) exists mainly in wild territory, areas not under extensive cultivation. The latter areas are the breeding places for the orange form. However, semi-wild red-or-white-clover fields provide excellent larval habitats and no doubt in some areas have provided better than normal habitats for it.

Richards ('31) gives the life zone distributions of these two races in the southern Appalachians. Apparently, the yellow form occurs at the higher elevations, being found in breeding quantities from Canadian life-zone to Transition and occasionally in Upper Austral. In lower Transition, the yellow is commoner. Below Transition, the orange is commonest but apparently it, too, is rare below the Upper Austral.

Clark (MS) states of the occurrence of the yellow form in Virginia: "Found in open country generally, especially in clover fields; formerly abundant throughout, now commoner in the higher mountain pastures and frequent in the southwest, elsewhere infrequent or rare, having been almost completely replaced by the western _eurytheme_. Though less common, it is still numerous in the southeast. Apparently a somewhat hardier butterfly than _eurytheme_ and better adapted for life in rugged regions, it is still predominant in the higher mountain pastures and still frequent in the valleys among the southwestern mountains."

From the Central Plains west, that is, in the part of North America with a more arid summer, the yellow form is restricted to mountain or valley meadows or to irrigated clover pastures. The orange form is found everywhere, on dry hill or mountain slopes (_Astragalus_, etc. as food), in meadows, mainly as migrants (clovers as food) or in alfalfa fields in the valleys. The yellow form is not found in alfalfa fields in abundance except when white or red clover is grown along with the alfalfa. It is in fields of mixed clover and alfalfa that the most extensive intercrossing between these forms occurs for here the breeding populations of each are in ecologically the same place. Two populations where these conditions are present were studied in the summers of 1941-42 (Hovanitz '43).

Edwards ('68-'93) believed that the planting of red clover as a forage crop westward into the Mississippi Valley during the nineteenth century was allowing the range of the eastern yellow-race to be extended westward. Scudder ('89) does not believe this correct, but rather that the planting merely allowed the butterfly to become more common as well as to occupy more territory. According to Scudder, it was very common in central Missouri in 1857. The yellow-race has certainly had its potential range extended by the planting of white and red clover throughout the Great Basin in irrigated pastures. According to Edwards, the yellow-race was supposed to be getting commoner about Lawrence, Kansas, but then (about 1880) did not yet equal the orange eurytheme. This was likewise true of Nebraska. It may be noted that this was just the time when the growing of red clover (the food plant of the yellow form) was beginning to receive competition from alfalfa (later the primary food plant of the orange form).

Early records for the yellow race in the west are often not available due to the idea that it was merely a color phase of the orange and therefore always occurred with it. This, however, is not true. Food plant, ecological and distributional data in most recent publications are not applicable to this race for the most part. However, very old yellow material from eastern California (middle 19th century) shows that it is not a recent migrant into the Great Basin.

Clark ('41) separates as a yellow phase of the orange form in the vicinity of Washington, D.C. individuals which have no orange pigment, but in shape of wings, manner of flight and other habits are similar to the orange form. He states (MS) that in 1937 "together with the orange form there appeared unusual numbers of the clear yellow phase which up to this time had been rare. We found several mated pairs in which one individual was yellow and the other orange. Since that time the yellow phase has been frequent in all broods. Up to this time it had been a fairly safe assumption that all yellow individuals were philodice. With the appearance of the yellow phase of eurytheme in numbers this assumption is no longer valid; yellow individuals may be either philodice or representatives of the yellow phase of one or other of the forms of eurytheme. At present all over Virginia except in the higher altitudes in the mountains and in the southwest, most of the yellow individuals prove on capture to be eurytheme."

On the basis of the genetic study of similar interbreeding populations at Mono Lake and Round Valley, California, (Hovanitz, '43), the "yellow phase" mentioned by Clark is now believed to represent yellow race individuals, altered by intercrossing. An exchange of adaptive genes probably has taken place between the forms, which, however, have retained their essential physiological characteristics.

The yellow-race is divisible into five geographic entities which have received names (Hovanitz, '43). We need not consider these subdivisions here as this is fully covered elsewhere (Hovanitz, '43) and is not essential to the present discussion.

Summary: The yellow-race occupies a more extensive range than the orange-race, occurring from Alaska to Guatemala and from the Atlantic to the Pacific Oceans. Its peripheral range has probably been little affected by the alterations made by man in the ecological conditions of North America. However, genetic alterations in the race have taken place through intercrossing with the extended range of the orange race. Also an increase in actual numbers of individuals of this race has taken place by the increase in larval food provided by man's cultivation of clover crops.

The Relative Abundance of the Races in the Different Regions

Data on 6,072 museum or preserved specimens of *Colias chrysotheme* were obtained from widely scattered geographical locations through the courtesy of the owners or curators of the material. The specimens were segregated into orange, yellow and white phases, partly by the author and partly by the persons supplying the information. The full data with detailed localities and dates are too extensive for publication; therefore, a condensation is given (table 1). Many of the localities are shown as dots on the maps (figs. 1, 2).

The frequencies of yellow-race individuals to orange-race individuals in various geographical regions are indicated on the map (fig. 4). The upper numbers of each fraction indicated represent the percentage of yellow-race butterflies as compared with orange-race individuals for that area. It is at once seen that there is a general decrease of yellow from north to south. In each of three ways from north to south this regularity is illustrated:[4]

Cordillera	Central Lowland	Atlantic Coast
100	100	77
100	100	55
86	60	12
67	41	
6		

The Frequency of the White Females in the two Races and in Different Geographical Regions

Within each of the races, the white females exist at a higher frequency in the north than in the south. The museum data, however,

Table 1. The frequency in percent of the three color phases of Colias chrysotheme in North America. The yellow is the percentage as compared with orange; the total (N) does not include any white females. The white female percentage is compared with the total yellow and orange females in the area.

Geographical region	%yellow	N	%white	N	yellow subspecies
Far Noroht west	100	240	88	77	vitabunda
Alaska	100	142	95	56	
Yukon Terr., N.W.Terrs. N.W. Brit. Col.	100	98	71	21	
MOUNTAIN REGION	74	1992	14	635	eriphyle
Northern portion	86	658	7	201	
Brit. Col. (Central and southern parts)	89	261	10	91	
Alberta	88	101	4	53	
Idaho	92	172	6	35	
Montana	70	124	9	22	
Southern portion	67	1334	18	434	
Wyoming	57	186	12	43	
Colorado	62	366	12	93	
Utah	78	482	16	164	
Nevada	52	63	19	43	
Washington (eastern)	78	46	14	7	
Oregon (eastern)	27	33	53	19	
California (eastern)	71	158	17	65	
PACIFIC COAST	0	340	43	187	(none)
Washington (western)	0	6	67	3	
Oregon (western)	0	14	11	9	
California (northwest)	0	131	47	60	
California (southwest)	0	189	43	115	

Geographical region	% yellow	N	% white	N	subspecies
SOUTHEAST	6	449	43	209	hageni
Arizona	1	224	39	75	
New Mexico	26	85	61	44	
Mexico	1	140	37	90	
NORTH PLAINS	60	232	25	88	
Maitoba	58	142	19	54	
Sakathewan, North Dakota, South Dakota, Minnesota	63	90	37	34	
CENTRAL PLAINS	44	193	33	96	
Nebraska	44	86	30	36	
Kansas	38	16	67	3	
Missouri	38	78	35	49	
Iowa	62	13	20	8	
GREAT LAKES AND VICINITY	57	307	38	140	philodice
Ontario	44	63	41	17	
Michigan	37	67	40	42	
Illinois	64	118	30	63	
Ohio, Indiana, Kentucky, Wisconsin, Tennessee	80	59	56	18	
SOUTH PLAINS AND MISSISSIPPI VALLEY	6	164	42	52	
Arkansas, Oklahoma, Texas, Lousiana, Mississippi					
NORTHEAST	77	1061	33	514	philodice
Quebec, Nova Scotia, New Brunswick, Maine, Vermont	63	57	10	31	
New Hampshire	100	34	19	16	
Massachusetts, R.I.	85	46	37	19	
Connecticut	89	73	30	47	
New York	81	357	33	220	
Pennsylvania	73	430	35	130	
New Jersey, Delaware	67	64	43	51	

Geographical region	% yellow	N	% white	N	yellow sub.
CENTRAL ATLANTIC	55	359	22	119	philodice
Maryland	46	37	20	15	
Virginia	61	80	21	39	
West Virginia	86	56		5	
District of Columbia	33	98	21	38	
North Carolina	52	88	14	22	
SOUTHEAST	12	77	28	36	philodice
South Carolina, Georgia	14	49	32	19	
Florida, Alabama	7	28	24	17	
GRAND TOTAL NORTH AMERICA	58.5	5414	30.6	2153	

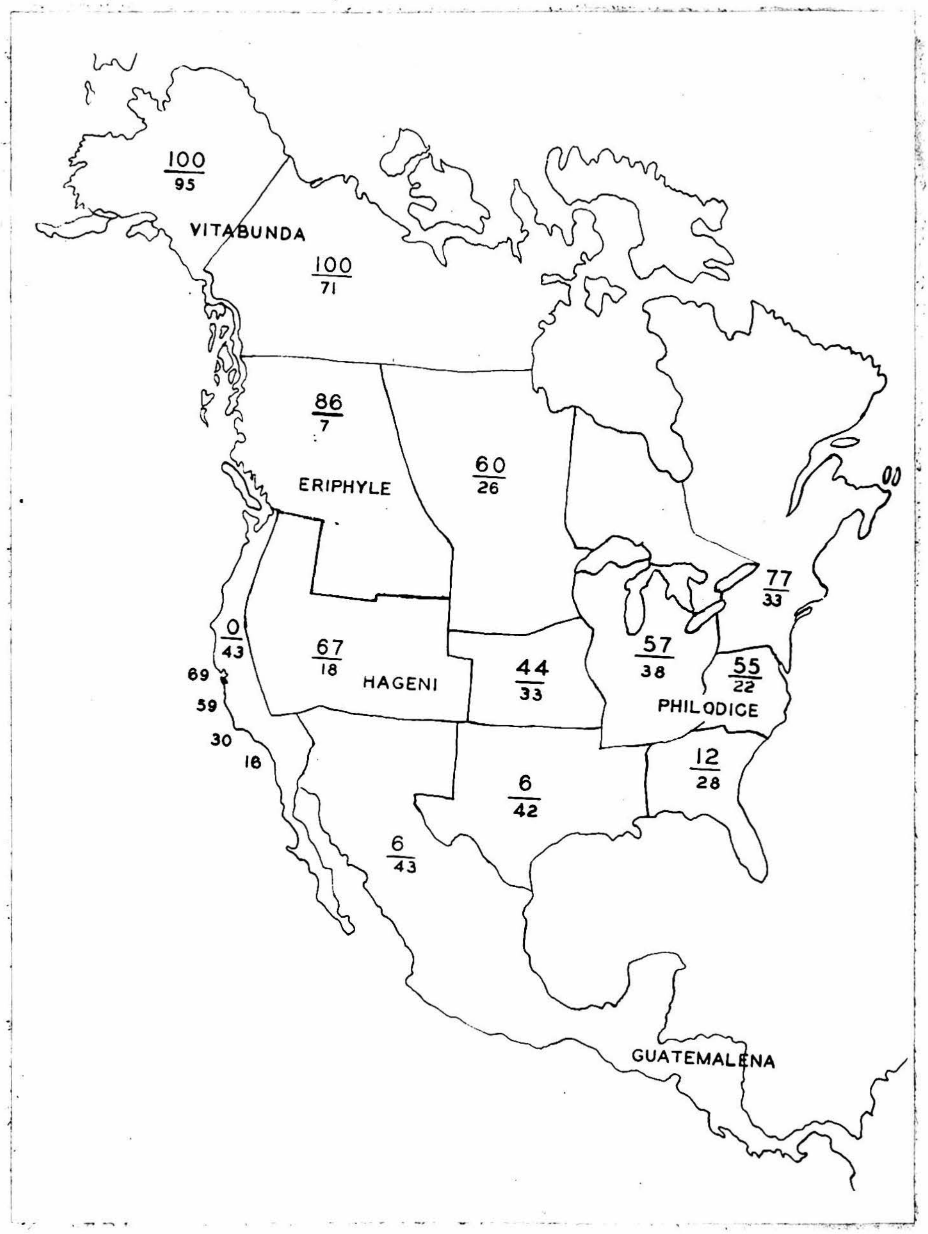

Fig. 4.- The comparative frequencies of the yellow to orange races in North America (upper figure in the fraction) and the frequency of white to normal colored females (lower figure in the fraction).

make no classification as to race of the white females. True frequencies within a race can be obtained only where the material is bred or in pure single race populations.

Data of the former type have not been obtained in sufficient numbers to give percentage values. Data of the latter type have been obtained in the Far Northwest where the yellow race is found alone, and in parts of California and Arizona where the orange-race is found alone. Prior to about 1925, such data could have been obtained in the Northeast on the yellow-race. In some localities, such as New Hampshire, even now the orange race is scarce enough to give negligible error to the data.

The map (Fig. 4) shows the frequency of white females throughout North America. The numbers shown as the lower figure of the fractions are the percentages of white females compared with the normal yellow or orange females in each area. The data is given in more detail in table 1.

Alaska has a white frequency of 95 per cent. Yukon Territory (and vicinity) has a white frequency of 71 per cent and illustrates the decreased frequency to the south. The Northern Mountain Region has a white frequency of 7 per cent, a tremendous decrease over the Yukon Territory. New Hampshire has a frequency of 14-19 per cent white females as determined both by random collection and by means of museum material (table 2).

Table 2.- The frequency of the white female in New Hampshire. Data for specific localities have been reorganized from Gerould ('23, '41).

Locality	% white		N
Hanover, N.H. 1911	16.1	±4.7	62
Hanover, N.H. 1923	9.8	±4.6	41
Goffstown, N.H. 1917	3.8	±2.6	52
Hanover, N.H. 1940	17.2	±2.4	244
Total	14.5	±1.8	399
New Hampshire (Museum material)	19.	---	16

The data for these areas of pure or nearly pure yellow-race are sufficient to illustrate the higher frequency of white in the north and the decrease to the south.

The orange race is found pure only along the Pacific Coast west of the Cascade-Sierra Nevade divide, Arizona and the lower portions of the Mexican plateau. Samples of significant size have been gotten in only one portion of the area - in California and Arizona. Museum material indicates an average frequency of 47 per cent in Northern California, 43 per cent in Southern California, and 39 per cent in Arizona.

However, more accurate data are available. These have been obtained by random sampling of populations from central California to western Arizona. Averages from four sections of this range give 69 per cent in the north through 59 and 30 per cent to 16 per cent in the southeast. These frequencies are shown in their approximate geographical location on the map (Fig. 4).[5]

The trend of the variation is the same in each of the orange and the yellow races. However, the yellow-race frequency is higher farther north than in the orange-race. For example, whereas a frequency of 70 per cent white is found in the yellow-race north as far as the Yukon Territory, in the orange race, 70 per cent is found much farther south in the latitude of central California.

Where the two races occur in the same area, the frequency of white would be the combined frequency in each of the orange and yellow races. It would follow that this would fluctuate depending upon the relative abundance of the two races in the area. The more abundant the orange race in an area, the higher would be the frequency of white. This is best shown by a diagram (Fig. 5). The percentages to the right of the middle line represent the combined frequencies of

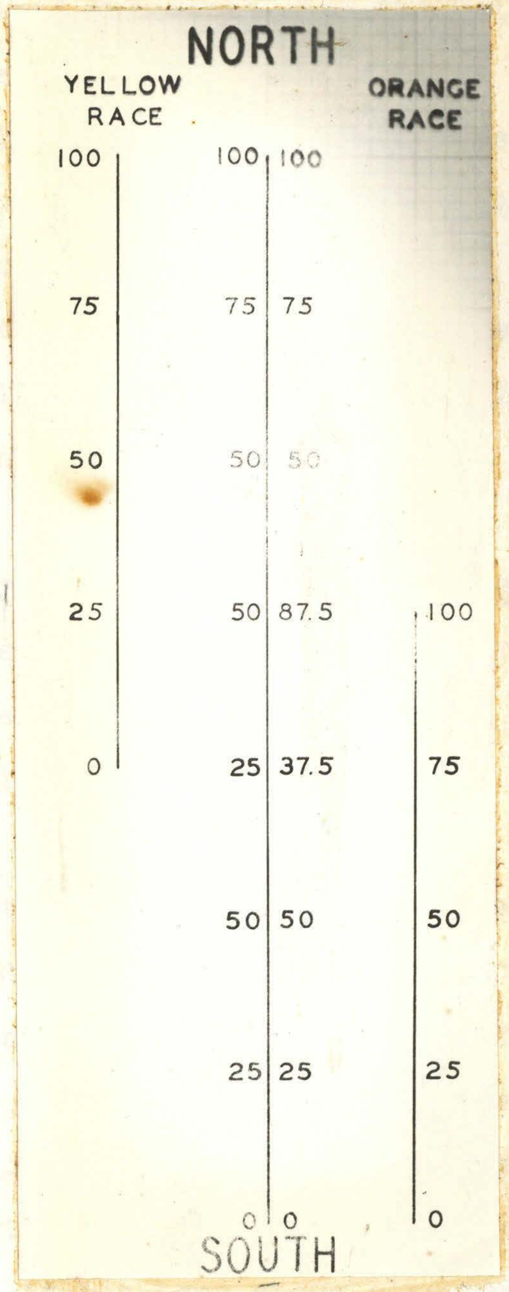

Fig.5. Diagram illustrating the results of intermixture of the yellow and orange race frequencies.

white when the two races are equally abundant; the percentages to the left are the combined frequency when the yellow race is twice as abundant as the orange.

The results of this intermixture of the racial frequencies will be as follows: The white frequency will begin high in the north and decrease to the south. As the abundance of the orange race increases, the white frequency will stop its decrease southward, reach an equilibrium and then increase. This increase should be proportional to the orange race increase (Fig. 5). The map (Fig. 4) shows this relationship in the geographical data.

Theoretically, the frequency toward the south should again decrease after the second "high". The data are not sufficiently abundant in the southern locations to show this clearly except in the pure orange locations in California.

Discussion

The different geographical distributions of the three color phases of Colias described above are found to be consistent with some adaptive qualities possessed by each color form. These adaptive qualities enable the particular form to exist better in the particular range occupied; apparently, the color difference is incidental to the main adaptive traits but directly related to it in growth metabolism.

For example, it has been shown elsewhere (Hovanitz, '42, '43) that the yellow and orange races differ in a series of physiological characteristics. These are related in the following ways to the characteristics of the environment in which the races are found.

A genetic diapause in the yellow race taking effect upon the larvae will enable this race to overwinter in a dormant state easier in cold territory than the orange race. The latter does not possess a diapause; hence, it is more likely to be killed by freezing. The range of the yellow race into the parts of North America with cold winters is consistent with this genetic trait (such as the far north, the high mountains and the east).

Likewise, the sterility and high mortality of the yellow-race larvae when bred on alfalfa and similar legumes would not encourage the race to expand its distribution with the increased growth of that plant, such as is the case with the orange-race. The dry summers and warm winters of the southern Pacific Coast are not desirable conditions for the growth of perennial Trifoliums, the food plants of the yellow-race.

Data on the better ability to survive under various conditions of the physical environmental factors are more difficult to obtain. However, breeding data (Hovanitz, '43) suggest that the orange-race is better able to withstand high temperatures than the yellow-race. This also follows from the lower life-zone distribution of the orange-race throughout the southeast and in its much greater abundance in the southern areas in general.

The northern distribution of the white female in the two races suggests that it has an advantage over the normal orange or yellow in those areas. The experimental evidence here is complicated and will be considered elsewhere (Hovanitz '43).

Summary

1. The North American distributions of two physiological races of the butterfly, Colias chrysotheme, have been described from an ecological and genetical point of view.

2. The orange-race has extended its range everywhere with the growing of alfalfa, but most especially in the eastern United States. The range in the western parts of North America has been altered only by the shifting of population abundance from wild areas to the alfalfa

fields in the valleys. The present area now extends from southern Canada to southern Mexico and from the Atlantic to the Pacific. Before about 1850, the eastward distribution ended in the Mississippi Valley. The occupation of the Northeast has been from a west and south direction. Apparently, the occupation of the new territory has been accompanied by a genetic acclimatization in the race caused either by environmental selection or by intercrossing with the yellow-race.

3. The yellow-race has essentially the same range now as it had before the presence of European man in North America. However, its abundance has been increased within those parts of the range where white and red clover are grown. The recently migrant but now resident orange-race has apparently changed the genetic adaptability of the yellow-race in a once pure "yellow" territory.

4. The range of the yellow-race is greater than that of the orange, existing farther north and farther south. Museum material indicates that the yellows are commoner than the oranges in the north.

5. The variation in frequency of a white genetic mutant existing within each race has been described. In each race, the frequency is highest toward the north. At a given latitude or ecological equivalent of it, whites are more frequent in the yellow race than in the orange.

6. The differences in the ecological distribution of the orange and yellow are shown to be due to the genetic physiological differences between the races; namely, a potentiality diapause for winter hibernation in the yellow race, a different food plant distribution in the two races and different physical environmental preferences.

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Footnotes

[1] Clark (MS) has suggested that the orange-race may be indigenous to the Carolinas. However, because of the scarcity of this form in early eastern material as well as the fact that it was unknown until described from California, it is not believed to have been there in any breeding quantity till after the middle nineteenth century.

[2] According to Clark (MS), Scudder was in error here as Smyth has found them very common around Charleston, South Caroline, as early as 1874 and 1876. The records collected for this paper indicate the accuracy of Clark's notation since orange forms were probably common near Chapel Hill, North Carolina in 1886, three years before Scudder's note was published.

[3] Alfalfa or lucerne seems to have had its origin in the steppes of Central Asia from whence it moved into the semi-arid Asia Minor and southern Europe. It was introduced into America by the Spanish explorers in Peru and Chile (possibly also Mexico). In the 18th century, there were some fields in production along the Atlantic seaboard under the name lucerne but these did not thrive, owing probably to the lack of inoculation for nitrogen-fixing bacteria. The first thriving populations were in California and thence these moved eastward to the Atlantic as described in the text. Other species of *Colias* are now pests on alfalfa in other regions. *Colias lesbia* flies in droves over alfalfa fields in Argentina just as *eurytheme* does in California. (*C. lesbia* may represent an isolated population of the orange-race.)

[4] Error in obtaining these figures is largely minimized by the wide distribution of the material in various collections throughout North America. Nevertheless, it is expected that collectors may have selected the rarer color phases in any locality. This would tend to minimize the differences between the figures rather than to increase them. Also, any tendency on the part of collectors to have an equal

representation of the colored types for purposes of uniformity in collections would decrease rather than increase the differences observed.

[5] Details of this work will be published separately.

The Distribution of Gene Frequencies in Wild Populations of Colias.

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Nearly all butterflies of the genus Colias have dimorphic female forms - the normal yellow or orange and the "albinic" or white form. The white form in both races of Colias chrysotheme in North America is controlled by a dominant autosomal gene which has effect only on the female sex (Gerould '23, Hovanitz '43a). This seems to be true also for Colias myrmidone and edusa = crocea of Europe and the christina complex of North America (Gerould '23, Ford '41 and Hovanitz '43a). The general distribution and abundance of the white female form of chrysotheme in North America has been described in detail (Hovanitz, '43b).

Owing to the ease of segregation of the normal and white forms, this material is excellent for the study of gene frequency variations and its biological causes in wild populations. For this purpose, samples of the populations of the orange race were obtained seasonally and geographically in 1941-42.

Method

Counts of normal and white females were made throughout the alfalfa growing districts of central and southern California, southwestern Arizona and the western Great Basin. The earliest samples obtained were by actually capturing individuals; later, however, counts alone of free-flying individuals were found to give larger numbers and more significant results.

Knowledge of the exact viability of the three genotypes expected in the populations in all locations is necessary before pertinent gene frequency data can be calculated according to the standard formula: $P^2+2pg+q^2$ (Hardy '08). As the experimental data suggest that the viability of the dominant homozygote (q^2) may be zero under certain conditions and equal to or better than that of the other genotypes under other conditions, the application of this formula under known conditions of viability is not possible. The data are therefore discussed not as gene frequencies but as phenotype frequencies only.

With no difference in viability between the genotypes, the gene frequency in a population will remain constant, with random fluctuations only (Wright '32, '42). With complete lethality of the dominant homozygote which is probably true in hot environments and in southern regions, the white allele will be eliminated at a rate as given in the accompanying table (Table 1).

The white allele has a selective value under certain environmental conditions which is greater than that of the normal yellow or orange allele (Hovanitz '43). According to the conclusions derived from museum data alone, the white allele should be at an advantage over the normal allele in the north as compared with the south. Therefore, in this detailed analysis of populations, a similar north-south rule should be found. The allelic frequency should vary directly with the change in climate between regions if the two are thus interrelated. In a previous paper (Hovanitz '42), it was stated that only by a polygenic or multiple allelic hereditary mechanism can a gradient of morphological, adaptive variation coincide with a gradient of

Table 1 . Table showing rate of elimination from a population of a dominant allele which is homozygous lethal, population size being at infinity. Assuming equal viability for the other two genotypes, the elimination will be at the rate shown by the drop between generations.

Frequency of white females in adult population	dominant allele frequency	generation
Ww	W	
66.7%	33.3	1
50.0%	25.0	2
33.3%	16.7	3
28.6%	14.3	4
25.0%	12.5	5
22.2%	11.1	6
20.0%	10.0	7
18.2%	9.1	8
16.5%	8.3	9
15.4%	7.7	10
9.1%	4.6	20
1.96%	0.98	100
.199%	0.099	1000
.000199%	.000099	1,000,000

changing environmental conditions. When this kind of hereditary system is not available, similar results can be achieved by varying ratios of two alleles with extremes of 100:0 and 0:100.

The data presented here illustrate the correlation between the gradient formed by the frequency of the two white alleles, and the gradient formed by the climatic conditions of the territory. They also show the effects of migration and random population change upon the gene frequency in various environments. A differential development rate of different genotypes and its effect upon population differences is noted in two populations.

The Frequencies of the White Female in California Populations

The frequencies of white females in California populations of the orange race are shown on the map (fig. 1). The general trend of highest frequencies in the North (69% white) and lowest frequencies in the South (13% white) is apparent.

The region sampled is divided into five district provinces by the local topography of the land. There are from north to south:

1. The San Francisco Bay area in the northwest.
2. The San Joaquin Valley area along the central portion of the state.
3. The Great Basin, represented on the map by Mono Lake and Round Valley.
4. Coastal Southern California, represented by scattered alfalfa fields throughout the coastal valley.
5. Coachella-Imperial-Colorado River Valley area of the southeast.

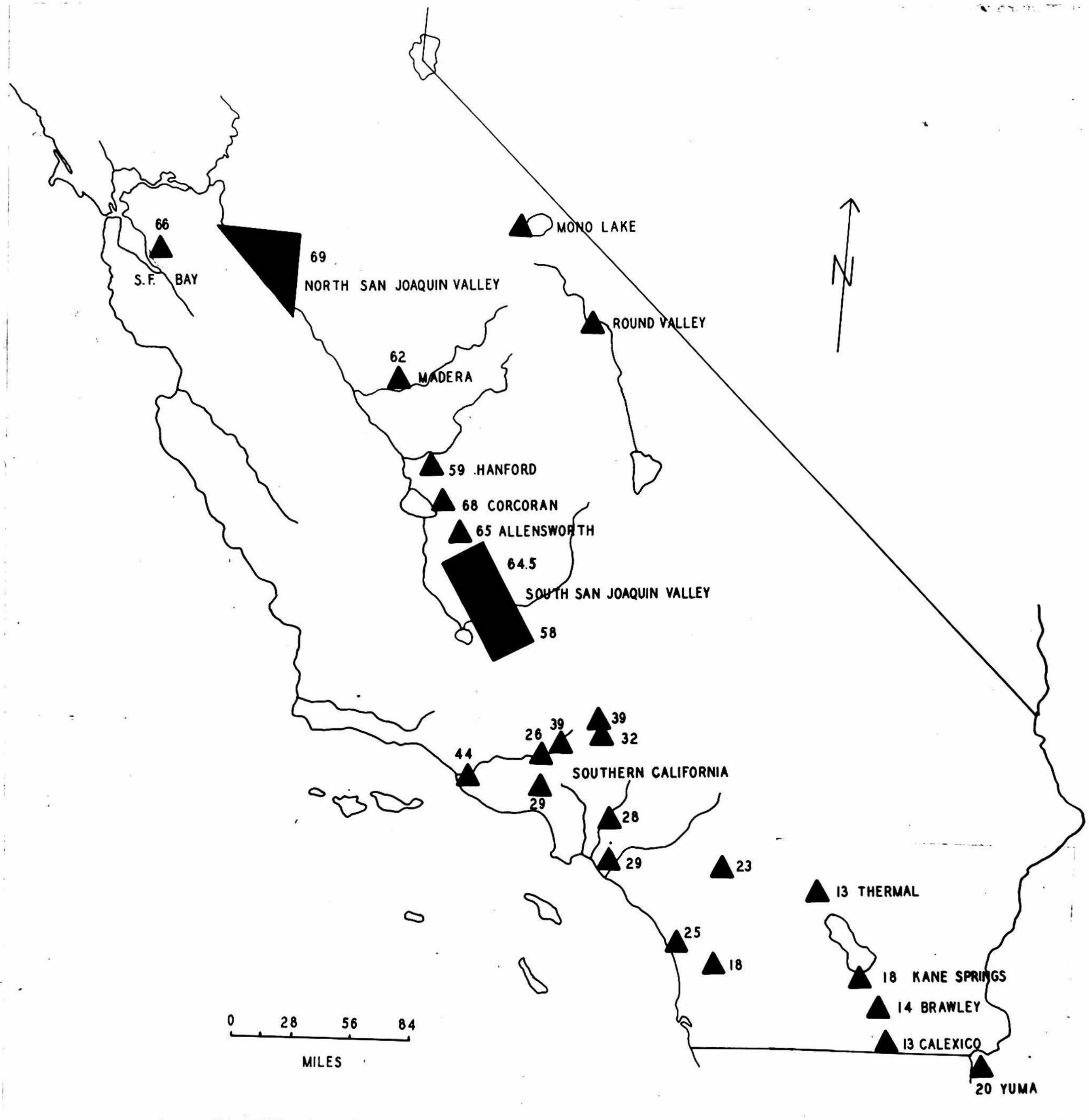

Fig. 1 .-- Map showing the locations in California and south Arizona of the populations sampled for white gene frequency and population structure of the races.

The genetic nature of wild populations are only studied or visualized with the aid of maps, climatological information and a knowledge of the local topography.

To neglect this data would be to eliminate the causal factors in the population differentiation; the cause of the differentiation is the point of this study.

The population differences (seasonal and geographical) are described below, starting with a central point, the coastal Southern California populations.

The Coastal Southern California Populations

The coastal Southern California populations sampled are shown on the map (fig.1) by numbers derived from the white frequency at each place.

The San Gabriel Valley area: The localities known collectively as the San Gabriel Valley area are situated at the lower end of the valley in the vicinity of El Monte, Garvey and Puente. The locality is marked on the map (fig.1) by the number 30. The populations are split into two parts, separated by the San Gabriel River, the centers of which are about five miles apart. Unless specified, samples were obtained generally over the area.

The general trend of the frequency variations during the 1942 season is given on the graph (fig.2) as"S. G. Valley." It is seen that there is little fluctuation during the season. A summary of the data according to the season for 1941-42 is given in Table 2. There are no significant differences in any ~~one~~ of the seasons, nor between the two years 1941 and 1942. Were the population number reduced to an exceedingly low value, it might be expected that

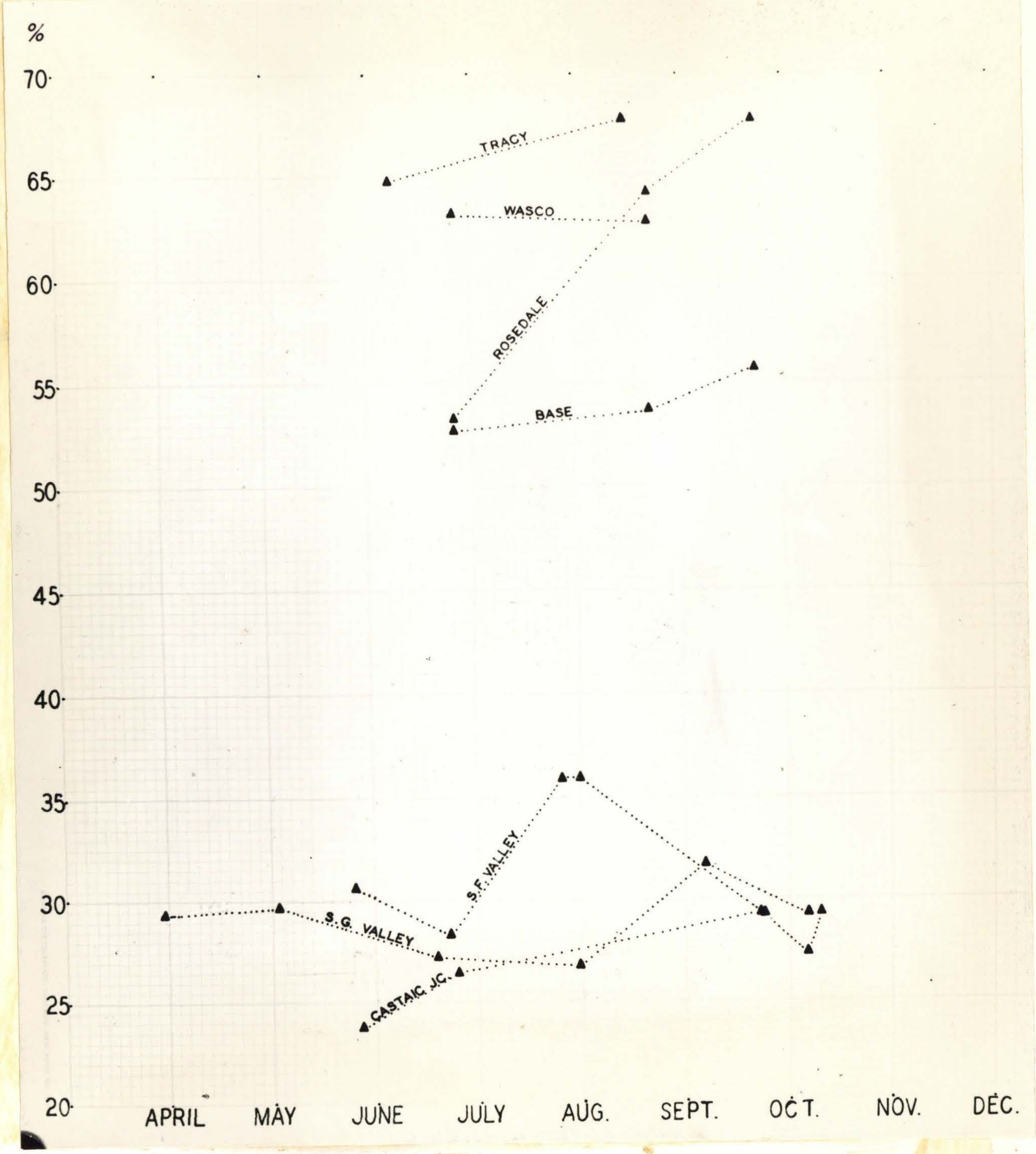

Fig. 2 .- Frequencies of white females during various seasons at various localities in California. Compare with Mono Lake fluctuations (fig. 6).

Table 2.- Seasonal frequency of white females in the San Gabriel Valley for two years.

Season	% white♀♀	N
1941		
Spring	21.62 ± 6.77	37
Autumn	30.03 ± 1.45	999
Total	29.73 ± 1.42	1,036
1942		
Spring	33.33 ± 7.27	42
Early summer	28.15 ± 1.55	906
Late summer	33.37 ± 1.65	812
Autumn	28.75 ± 1.82	612
Total	30.19 ± 0.94	2,372
Grand total	30.05 ± 0.78	3,408

random changes in gene frequency would occur, but no such variations are present. Apparently the population size is very large, or the environmental selection for the white and orange alleles is rather closely regulated. Both are probably true.

In May and June 1942, separate data were obtained on each side of the San Gabriel River (Table 3) in order to determine if a difference might exist, due to the spatial isolation. This is a test of degree of population movement as well as size since a difference in the results would indicate that the area is not well mixed or that random changes have taken place in gene frequency.

In both May and June, the frequency was lower on the west side than the east side. In neither case was the difference equal to or greater than 2X the standard error. With the combined data, the difference is 5.80% ± 4.54% or considerably less than 2X the standard error of the difference.

<u>The San Fernando Valley Populations</u>: The San Fernando Valley populations are located about 30 miles northwest of the San Gabriel Valley. On the map (fig.1) the place is denoted by the number **30**. Between these two localities is located the city of Los Angeles and two "mountain ranges" (Santa Monica mountains and part of the "Puente Hills." Therefore, there are no alfalfa fields to unite the two places. The chance of much gene exchange across this 30 miles under normal circumstances would seem rather doubtful. However, the species is somewhat migratory and at times there is probably considerable exchange. The climate at the two places is very similar, being under the same influence of the prevailing westerly winds and ocean fog. There should be very little differential climatic selection.

Table 3.– Frequency of white females at two localities on the two sides of the San Gabriel River (in the San Gabriel Valley). 1942. The numbers are % white females.

	West side	N	East side	N	Total	N
May	27.27 ± 4.48	99	30.57 ± 3.21	229	29.51 ± 2.51	328
June	25.40 ± 2.10	433	33.10 ± 3.91	145	27.34 ± 1.83	578
Total	25.75 ± 1.89	532	31.55 ± 2.40	374	28.15 ± 1.55	906

The trend of the variations in this region is shown on the graph (fig.2) as "S. F. Valley." The variations in the populations of the San Fernando Valley are not statistically different. Even the July and August percentages of 35% white are not different from the average of 31.48 for this period, (Table 4).

During the period September 23 to November 7, tests were made to determine if there were a differential development rate between the two types of female. One particular field in the San Fernando Valley had a heavy infestation of Colias and was in full eclosion. Frequencies were obtained in this field at two day intervals of (a) percentage of white females freshly eclosed on that day and (b) percentage of white females free-flying in the population. Frequencies were obtained in local fields nearby to serve as a control.

The graph (fig.3) shows the variation in frequencies between newly emerged females in one alfalfa field (as judged by the fact that they had wet wings and were in copulation) and the frequency of free-flying females in the same place. There is also shown by dotted lines the frequencies in two control fields about one mile from the former place.

From a high of 36% white females which eclosed on September 23, the frequency dropped to 29% on each of the two succeeding periods of two days each to a low of 24% on September 29. The difference between the extremes is 11.87% ± 4.23% or nearly 3x the standard error of the difference (Table 5).

Two days following the sample of 36% of newly emerged females, samples were made of the frequency of free-flying individuals. On September 25, the frequency was 45%. This dropped at a rapid rate at first but was slower later until a low of 25% was reached on

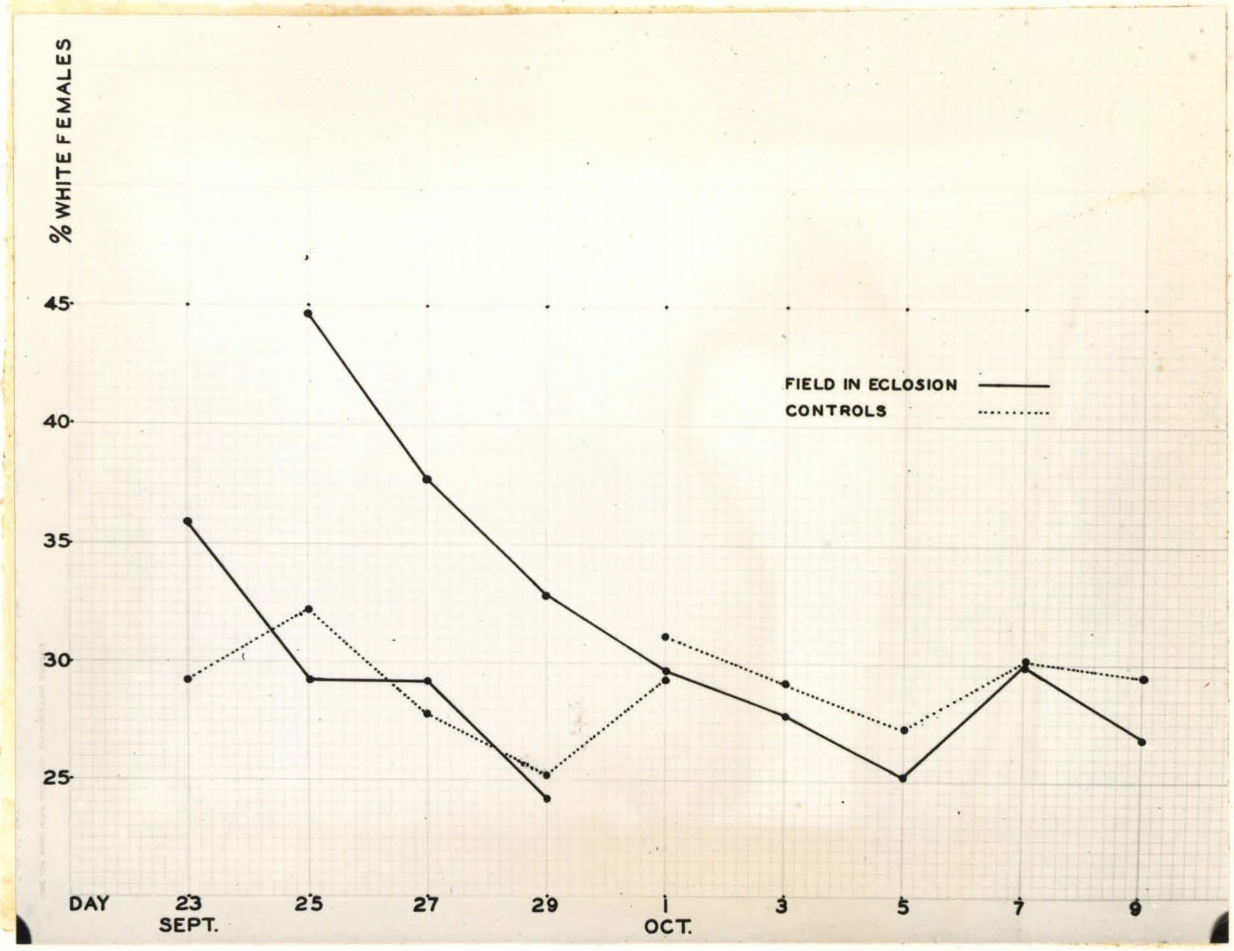

Fig. 3 .- Frequency of white females in a San Fernando Valley alfalfa field during an adult eclosion period. The lower heavy line represents freshly eclosed females with wet wings. The dotted lines represent controls in other local fields. The control first used was replaced by a second on October 1.

Table 4 .-- White female frequency during the season at San Fernando Valley, Calif. 1942.

Date	% white♀♀	N
May 25	30.57 ± 3.04	229
June 2 2	28.43 ± 2.01	503
July 25	35.97 ± 4.06	139
Aug. 1	35.96 ± 4.50	114
Sept. 7	17.95 ± 6.15	39
May- Sept. 7	30.37 ± 1.43	1,024
Sept.23-Nov.7 (see Table 5)	30.29 ± 0.43	11,149
Total	30.30 ± 0.42	12,173

Table 5.- The frequency of white females at a given alfalfa field in the San Fernando Valley during the course of an adult eclosion period. The copulated females were newly eclosed on the day of the sample. The comtrol samples were made in fields approximately one mile from the tested field.

Date	Free-flying, %w	N	Copulated, %w	N	Controls, %w	N
Sept. 23	-----	-	35.91 ± 2.96	323	29.24 ± 1.96	537
Sept. 25	44.58 ± 2.04	590	29.22 ± 2.36	373	32.22 ± 2.58	329
Sept. 27	37.61 ± 2.07	561	29.20 ± 3.06	226	27.81 ± 2.31	374
Sept. 29	32.85 ± 1.85	685	24.04 ± 3.01	208	25.12 ± 2.17	414
Oct. 1	29.59 ± 1.59	828	--------	16	29.45 ± 2.52 (31.05 ± 1.96	326 554)
Oct. 3	27.75 ± 1.76	645	--------		(28.61 ± 2.29	423)
Oct. 5	25.23 ± 1.46	880	--------		(27.40 ± 1.99	500)
Oct.7	29.97 ± 1.47	971	--------		(30.08 ± 2.40	359)
Oct. 9	26.90 ± 1.78	617	--------		(29.49 ± 2.57	312)
Nov. 7	37.76 ± 4.88	98	--------		--------	
Total	31.30 ± 0.61	5875	29.76 ± 1.35	1146	29.00 ± 0.71	4128

October 5. The difference between these extremes is 19.35% ± 2.50% or nearly 8x the standard error of the difference. Some of the consecutive samples are likewise more than 2x the standard error of the difference. For example, that between September 25 and 27 is 6.97% ± 2.93% or over 2x. The difference between September 27 and 29 is 4.76% ± 2.78% or just under 2x.

On October 7 and 9, the population seems to have leveled off to a standard range of fluctuation and after the ninth, individuals were scarce. As far as adults were concerned, the population size was greatest between September 23 and 27 when the alfalfa field was literally covered with them. At this period all individuals were exceedingly fresh. From September 27 to October 9, the population size decreased rapidly. Samples of newly emerged adults could not be obtained after September 29 owing to the very rapid drop in eclosions. By October 3, fresh individuals were rare and by the ninth nearly all were old and worn. The alfalfa field was being cut from about four days before September 23 and was completely cut on the twenty-seventh. At this period thousands of pupae were crushed. By October 9, the shortest average new growth in the field was about one foot high; females rarely lay eggs in a field higher than this.

The control samples were gotten in two fields about one mile east of the field being tested. The fluctuations in the control samples are not statistically different but they follow the experimental curves to a certain extent. There is a drop between September 25 and 29 following the drops in the other curves; also a ~~rise~~ drop ~~preceded~~ followed by a rise between October 1 and 9 following the same in the experimental curve. The control plots were in fields which did not have a high percentage of eclosing individuals. Their population was at least largely composed of individuals which had flown in from elsewhere. This was especially true of the field used between September 23 and 29. The controls are more representative of the valley populations as a whole than the experimental population. Two possibilities may account for the very rapid gene frequency change in the one field:

(a) the white individuals are faster in development than the orange so that there are more of them at the earlier stages of the adult eclosion, or

(b) the white gene has somehow gotten a rather high concentration in one place due to chance.

The former possibility seems more likely because it fits all the data better. One can account for the higher frequency at first in both experimental curves by assuming that more white females emerge at first. As more orange females would emerge late, it would be expected that the frequency of white females would drop below the average for the region, provided that there was a general exodus of adults from the area. The assumption of decrease in adults is correct because (a) the number of adults visibly declined from September 27 on, (b) adults were seen to be flying away from the field in large numbers, (c) the area already had a higher concentration of adults than any other place in the valley; therefore, more adults would leave than arrive, and (d) a percentage of the early emergenc~~i~~es would have died, thus lowering the number of adults there. As expected, the frequency of whites dropped below the average frequency of 30% to a low of 25%. In favor of the first possibility also is the drop in frequency in the newly emerged females.

The tendency of the control curves to follow the experimental ones can also be explained by the first possibility (a). Since adults were leaving the field and scattering in all directions, it may be assumed that the higher concentration of whites at the one place may influence those nearby.

To explain the second possibility, one must assume that a very rare chance concentration of white females had laid the eggs from which the brood arose. The number of females must have been huge considering the size of the brood. The larger the number, the less the chance of getting a concentration fluctuating from the normal. The size of the brood must have been in the millions.

On the basis of the second possibility, there is no way of explaining the drop in frequency in the newly emerged insects unless one assumes that one day a month before the emergence mostly white females had laid eggs in the field and a few days later a higher frequency of orange females laid the eggs. The likelihood of this being true seems nil.

The second possibility assumes that the drop in frequency is due to a mixing of the adults with the general valley population. This would entail a great movement of adults into the field. On the contrary, the movement was visibly away from the field. The frequency would not have dropped below the general valley frequency of 30% by this method, nor would it have dropped so quickly.

Therefore, unless the development rate of white females is faster than that of the orange, it does not seem likely that the change in gene frequency could have occurred.

Populations of the Santa Clara River Drainage Basin: Airline about 15 miles north of San Fernando Valley along the central part of the Santa Clara River Valley, three samples were made during 1942. This population (Castaic Junction) is located on the map (fig.1) by the number 26. The fluctuation during the season is not significant but varied from 25% white in May to 27% white in June to 29% white in September. This gradual rise is illustrated on the graph (fig.2) as "Castaic Jc." It is possible that the frequency has gradually risen during the season and it could easily have done so by migrations from the extensive populations in the San Fernando Valley. There is, however, a range of hills separating the two places (Santa Susana Hills). Comparing these two localities for the 1942 totals shows that "Castaic Junction" has a frequency which is statistically lower than that in the San Fernando Valley:

Castaic Junction	27.44% ± 1.38%	1,046
San Fernando Valley	30.30% ± 0.43%	12,173
Difference	2.86% ± 1.44%	

"Castaic Junction" is farther north than San Fernando Valley but has a much hotter and dryer climate owing to its sheltered location within the hills and far from the coast. The populations in the San Fernando Valley are at the westernmost and coolest portion of the valley. The inversion of the north-south "rule" in this case may be due to the climatic regularity or possibly to chance alone.

Down the Santa Clara River Valley from Castaic Junction toward the coast is a distance of 40 miles airline. Apparently there are few alfalfa fields between these two places and not very many even at the coast. However, a small sample was gotten at the latter place (Oxnard) with a frequency of 43.75% ± 6.20% white females (see map 1 at point marked 44). Climatically, the ~~air~~ area is cooler, foggier and more humid than any of the other Southern California locations. It should have a higher frequency of whites as is indicated. However, the small number renders the significance of the data doubtful. The frequency is apparently statistically different from the two closest populations by more than 2x the standard error of the difference: Difference from Castaic Jc. 16.31% ± 6.35% and from San Fernando Valley 13.45% ± 6.40%.

In one of the branches of the Santa Clara River Valley known as Bouquet Canon, a sample was gotten in May 1942. This place is about 10 miles east of Castaic Junction and is more or less connected with it by occasional alfalfa fields. The frequwncy found here in May 1942 was 39%. The number of individuals again was low and the frequency is, therefore, once again doubtful. Compared with the populations at Castaic Junction, the difference is nearly 4x the standard error of the difference for the month of May and about 5x for the seasonal average at the latter place:

		N
Bouquet Canon	39.34% ± 6.25%	64
Castaic Jc. May	23.64% ± 4.05	110
(Difference)	15.70% ± 4.55%	
Castaic Jc. 1942	27.44% ± 1.38%	1,046
(Difference)	11.90% ± 2.45%	

The frequency of 39% is very close to the frequencies in the Antelope Valley to be considered later and may owe its origin to that source.

The Antelope Valley Populations: The Antelope Valley is located about 25 miles east of the Bouquet Canon population. It is really the western arm of the Mojave Desert but has considerable agriculture due to the presence of water for irrigation. The northern arm of the San Gabriel Mountains extends between the desert and the coast here, but the elevation is very low, owing to the change in geological structure. Cold winds from the coast blow through the area causing it to be climatically quite different from other parts of the desert. In the summer it may be very hot or may be cold and windy; in the winter it is colder than the Southern California coast.

Samples were obtained in May and June at two places, Palmdale and Lancaster. Between these localities, a distance of 8 miles, there is no alfalfa so that the places may be considered as isolated by dry desert. At Palmdale in May the frequency was 39.44% ± 4.1% (N=142). In June it was 31.96% ± 2.72% (N = 291). In June at Lancaster the frequency was 38.83% ± 4.81% (N = 103). None of these differences approach statistical significance. The total for Antelope Valley for the season, 35.26% ± 2.08% (N = 536) is not different from the closest population, Bouquet Cañon.

At first sight it might seem that the desert should have a low frequency of the white gene. However, this part of the desert is cold in winter and does not always get hot in summer. In 1941, cold winds were almost continuous throughout the summer with only one or two "warm spells".

The Antelope Valley populations are the stepping stones to the San Joaquin Valley populations farther north with frequencies above 50%. The more coastal populations of Southern California are separated from the San Joaquin Valley by 70 airline miles of rugged mountains, while Antelope Valley is separated from it by only 20 miles airline across the range. The map does not illustrate the difference as the Lancaster-Palmdale areas are far to the southeast in the Antelope Valley.

Other Southern California Populations: Samples were obtained at four other coastal localities which are designated on the map (fig.1) by the white female frequency; namely, Santa Ana, 29%, San Pasqual-Escondido, 18%, San Luis Rey, 25% and San Jacinto Valley, 23%.

The Santa Ana population on the map is 20 miles airline directly south of the San Gabriel Valley samples. The area in between is nearly continuously strewn with alfalfa fields in the lower places. The climate is probably a little cooler in the summer and warmer in the winter as it is closer to the coast; however, this is only very slight. The white female frequency is identical with that of the San Gabriel Valley.

The San Jacinto Valley population is about 50 miles airline due east of Santa Ana (number 23 on fig.1). Owing to its inland location and higher elevation of 2,000 feet, it has a much warmer and dryer climate in the summer and colder in the winter. The white female frequency is lower here as is expected considering its climate and location. The significance of the difference between its frequency and adjacent populations follows:

		N
San Jacinto Valley	22.65% ± 1.82%	521
Santa Ana	29.41% ± 2.02%	510
(Difference)	6.76% ± 2.71%	
San Pasqual-Escondido	18.47% ± 2.19%	314
(Difference)	4.18% ± 2.84%	
Thermal	12.95% ± 2.85%	139
(Difference)	9.70% ± 3.78%	

The San Jacinto Valley-Santa Ana difference is just over 2x the standard error of the difference. The difference with San Pasqual-Escondido is nearly 2x while the difference with Thermal is almost 3x.

The San Luis Rey frequency (25%) is so much like others in the vicinity, and the number in the sample is so low that it is hardly different from any adjacent population. The place is located on the coast about 50 miles southeast of Santa Ana. The climate is very similar to that place.

The San Pasqual-Escondido population is located inland about 15 miles southeastward of San Luis Rey. The climate is apt to be warmer than the latter place or Santa Ana and quite similar to San Jacinto Valley. We have seen that the frequency is about 2x the standard error of the difference in comparison with the latter place. It is close to or identical with the frequencies in the Imperial-Coachella Valley and quite different from Santa Ana and San Gabriel Valleys. On the coastal side of the mountains, it is the lowest frequency known and is likewise the most southern known.

The Coachella-Imperial-Colorado River Populations

The Coachella-Imperial-Colorado River Valley is relatively isolated from the other populations considered before by the mountain ranges which include the San Jacinto range, the Santa Rosa range and the Laguna-Cuymaca mountains. This region is a region of exceptionally low rainfall, receiving far less than 5 inches annually. It must have been completely uninhabited by *Colias chrysotheme* before the period of irrigation, due to the lack of any larval food.

The valley floor was covered only by shrubs typical of the Coachella and Colorado deserts before irrigation. The area is largely below sea level and all samples aside from Yuma were obtained below the level of the sea. At Kane Springs, the elevation is about -200 feet. A few alfalfa fields exist north of the Salton Sea at which place the sample at Thermal was obtained. Between Thermal and Kane Springs the area is almost all barren desert rock or sand and the water surface of the Salton Sea; this is a distance of about 50 miles. From Kane Springs to Calexico on the Mexican border the area is commonly continuously covered with irrigated alfalfa fields. Climatic conditions are mild in winter so that the populations can be breeding all year. In summer, the temperatures rise to 130° F. at times; occasionally, for periods of one or two weeks constantly the temperatures day and night will not drop below 100° F. Such continuous temperatures are sufficient to sterilize the butterflies of the coastal population (at least with a high humidity). Possibly there has been established a physiological race able to withstand such conditions, or perhaps the low humidity of the region allows cooling by evaporation.

The white female frequency in this area is the lowest known, varying from 12.9% to 17.8%. The differences in the populations to be described are hardly to be correlated with climatic differences though such correlations are yet to be made accurately. The valley is climatically very similar from the Mexican border at least to Indio. Farther north, cooling by winds from the San Gorgonio Pass takes place.

The frequency at Thermal, 12.95% ± 2.85% is different by nearly 3X the standard error of the difference from the nearest "coastal" population at the San Jacinto Valley. They are separated by a mountainous area about 50 miles airline distance and in which there are no alfalfa fields. The butterflies breed in the mountain meadows in the summer, however. The next nearest population sampled is at Kane Springs which is not significantly different from it: 17.80% ± 149% (N = 663); difference = 4.85% ± 3.57%.

The Kane Springs frequency of 17.8% is obviously not different from the next population about 15 miles south, Brawley, 14.48% ± 2.06% (N = 290). Brawley in turn is not different from Calexico about 20 miles farther south, 12.99% ± 1.57% (N = 462). The extremes, Kane Springs and Calexico, are different by more than 2X the standard error of the difference 4.81 (4.8% ± 2.16%).

It should be noted that there are almost continuous alfalfa fields from one place to the other and that the distance is but 35 miles.

The average for the Coachella-Imperial Valley is 15.32% ± 0.91% (N = 1554) which is obviously very different from the average for all of coastal Southern California (including Antelope Valley) of 29.78% ± 0.32% (N = 19,050).

Along the portion of the lower Colorado River just south of Yuma, Arizona, on the east side of the river, there is a considerable extent of irrigated agricultural area. Alfalfa fields are strewn throughout the area from Yuma to San Luis on the Mexican border. The sample of the Colias population here was gottem in various fields along the 15 mile airline distance rather from any particular field (fig.1).

The climatic conditions in this area are the same as in the

Imperial Valley and it would be expected that the white female frequency would be either the same or lower. This place is about 40 miles airline east of Calexico but this distance is barren, waterless desert of moving sand dunes and partly creosote-bush vegetation. Without flowers for nectar, adults could hardly travel this distance in the dry atmosphere, especially when it is so hot. In the winter mild weather, however, an exchange of adults may take place. A female adult which was well fed as a larva will live for two or more very active days with no food or water and be capable of laying a hundred or more eggs even with no more food.

The frequency of white females in the Yuma-San Luis area is 19.54% ± 1.91% (N = 481). Compared with the Imperial Valley at Calexico, this is 2½X the standard error of the difference (Diff. = 6.55% ± 2.37%).

It was expected to find the frequency in the lower Colorado River area to be lower than the Imperial Valley. However, the approximate frequencies derived from the museum material (Hovanitz '43) shows that Arizona as a whole has a frequency of about 39% white. Nearly all these data are from the portion of Arizona at the higher elevations. Locations closer to the latter region would be expected to have a frequency varying toward it provided there is some population interchange. Migration could easily take place up and down the Gila River, for alfalfa fields tend to follow it. This river connects the Yuma area with the Phoenix agricultural area where alfalfa is very commonly grown and where the fields are heavily infested with Colias (Wildermuth, '14).

Including the Yuma area in the total, the southeastern region on the map (fig.1) has a frequency of 16.31% ± 0.82% white females

(N = 2,035).

The San Joaquin Valley Populations

On the map (fig.1), the San Joaquin Valley lies along the central strip of the state between the places marked North and South San Joaquin Valleys. Actually the San Joaquin Valley is the larger and southern portion of the Great Valley of California which is continuous from the south at the Tehachapi mountains to the Trinity-Cascade ranges in the north. The climate in the San Joaquin Valley is "mild" the year round. Summer days are normally hot (above 100°C.) but the nights are cool. Winters are cool but not cold; frost forms for 2 or 3 months of the year especially to the north. Rainfall is highest in the north (from 10 to 20 inches) and lowest in the south (about 5 inches or less). Temperatures are higher in the south than in the north but the humidity is lower in the south than in the north. Alfalfa is grown very extensively in the valley, especially in the region at the northwest (at the big triangle on the map), at scattered areas throughout the central part and very commonly in the southern region near Bakersfield (at the rectangle on the map).

The white female frequency throughout this area is over 50% as compared with about 30% in southern California. The two zones are separated by a mountain barrier which is, however, not barren of Colias populations. The individuals are merely more scattered as they must have been over the entire west before alfalfa was grown. The population size must be relatively small; consequently, the gene frequencies would be controlled primarily by migrations from the adjacent alfalfa fields. The population size in the alfalfa fields of California is so huge at the present day that, except in some very isolated places, the gene frequency is probably controlled by them everywhere.

On the map (fig.1), the general white female frequencies can be observed, those rising from the southern part of the San Joaquin Valley at 58%, to 69% in the north.

The South San Joaquin Valley Area: Samples were made in this area at points about 6 to 10 miles apart. These data are too detailed for the large maps and the area represented thereon by a rectangle is shown enlarged (fig.4). The rectangle represents an area about 60 miles long in a northwest-southeast direction and about 25 miles wide. Alfalfa fields in this area are located in relation to the distribution of the sampled populations. However, there are more fields in the north than the samples would indicate. Near the limits of the rectangle in the southeast and southwest the alfalfa fields are isolated plots surrounded by unirrigated desert. This is true to a certain extent in the central and northern parts, too, but here also there is irrigation of sugar beet farms, beans, etc. Just north of Bakersfield and running southwest between Rosedale and Panama is the Kern River. Its flood basin occupies a wide area in which there are no alfalfa fields. The river itself is no barrier. The area to the north and west of the rectangle is partly dry, unirrigated hardpan soils and partly agricultural. There is no sharp delimitation of the alfalfa-Colias populations in either the west (north of the Kern River), the north, or the northeast. However, the zone directly north of Bakersfield and east of Famoso and Shafter is largely unirrigated lands left in semi-desert state. Completely around the east side of Bakersfield, Arvin, Weed Patch

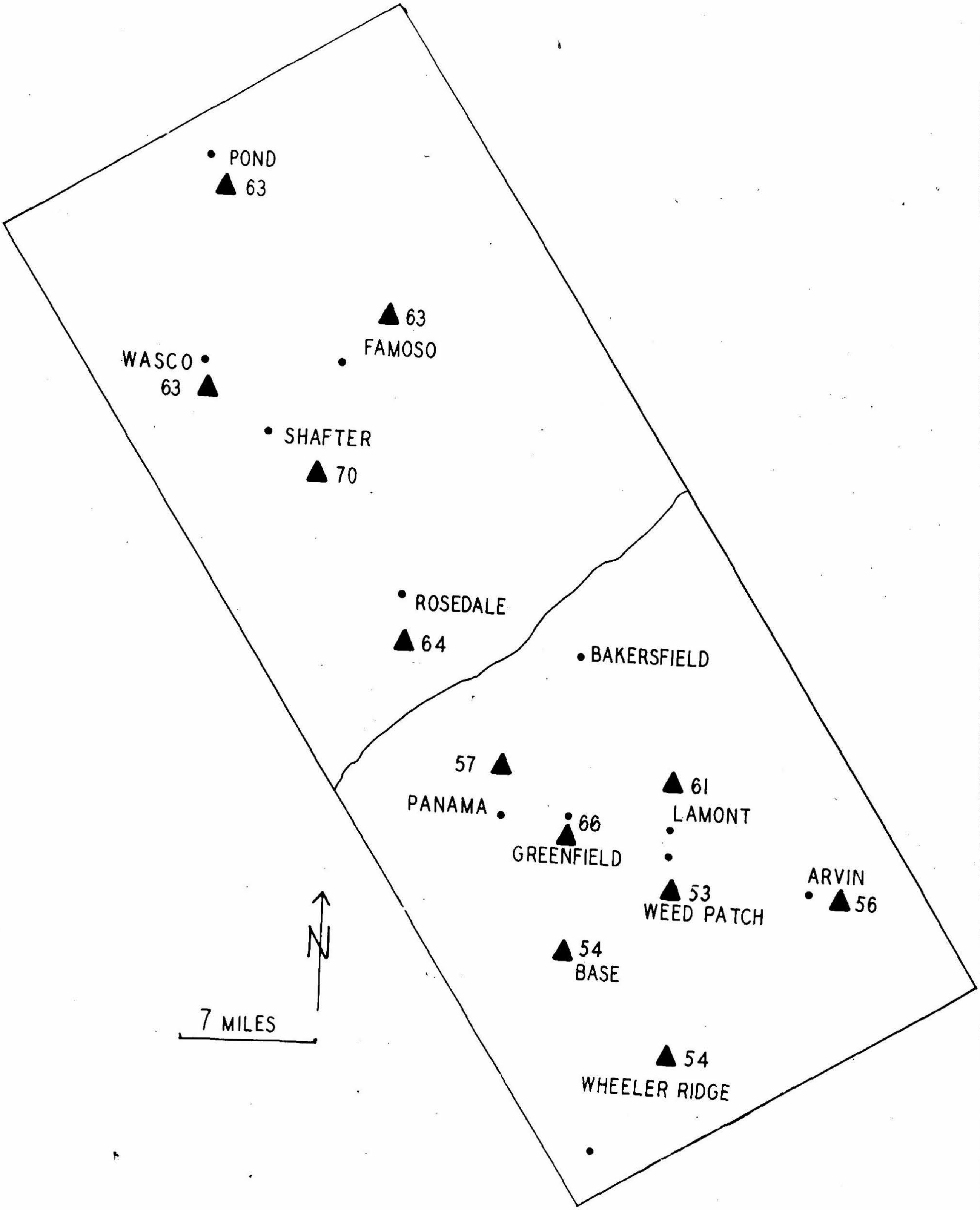

Fig. 4 .- South San Joaquin Valley area represented on the map of California by a rectangle. Dots are locations of towns and triangles are locations of alfalfa field sampled. Kern River runs through center.

and toward Wheeler Ridge, the area is devoid of alfalfa. Much of it is devoid of irrigation. West of Base and Wheeler Ridge, alfalfa is occasionally present, but not too commonly. This spotty distribution of breeding places for Colias is of signal importance in understanding the rapid change in gene frequencies which take place.

The frequencies for the San Joaquin Valley placed on the map (fig.4) are those obtained on August 21, 1942. Three localities were sampled earlier, on June 25, 1942 and two of these later on September 22, 1942.

The three population samples in June are from north to south:

		N
Wasco	63.19% ± 1.08%	1,964
Rosedale	53.33% ± 1.31%	1,440
Base	52.68% ± 1.28%	1,528

Wasco is obviously quite different from either Rosedale or Base. Being in the north, it is expected that it would have a higher frequency than either of the latter two. However, as the distance is small, chance variations within sub-units of a population could be expected to alter the significance of the north-south rule. In climate, Wasco is but slightly if any different from Base or Rosedale. It might have been expected that Rosedale would have had a frequency exactly intermediate between Wasco and Base. It is geographically located exactly between the latter two (fig.4). This was not the case for though it was slightly higher than Base the difference is not significant even with the large sample.

At the later date (August 21), two months later, these same localities were again sampled. The frequency at Base has remained essentially the same (fig.4); the frequency at Wasco has also remained the same. Rosedale, however, has risen and is now equal to Wasco rather than Base:

	June	N	Aug.	N
Wasco	63.2%	1,964	62.9%	942
Rosedale	53.3%	1,440	64.2%	812
Base	52.7%	1,528	53.7%	389

The change in gene frequency necessary to alter the phenotype frequency this much is great. The two months between the sampling dates is long enough in the vicinity of Bakersfield at that time of year for two generations. Therefore, sometime about the 20th of July there was a flight of adults which laid the eggs from which came the August 21, adult flight. Possibly at this time there was a movement of adults southward sufficiently to alter the gene frequency. The population size itself is so enormous that the change could not have come through chance. If selection were the causal factor, it too would have had to be very great. Assuming equal viability of the three possible genotypes, the gene frequency for a population of 50% white would be 33.5% dominant alleles and for 66.7% would be 44.4% dominant alleles. The selection could have occurred against or for any of the three genotypes. Were the homozyote dominant completely lethal, the change from 50% to 67% could be accomplished in one generation (see table 1), providing selection against the two remaining genotypes was equal. The former probability of mass movement or shifting of the adult population is considered the most likely explanation for the change (fig.2), especially considering the spotty distribution of breeding places.

The distribution of frequencies in the areas adjacent to these latter three places is a good key to the verification. Arvin, Weed Patch and Wheeler Ridge are the western and southernmost localities adjacent to Base. They are 56%,53% and 54% respectively. North and east of Base are Lamont 61%, Greenfield 66% and Panama 57%.

The latter two are exactly intermediate to Rosedale. The frequency of Panama is closest to Base but the locality is closest to Rosedale. The reverse is true for Greenfield. It is possible that a flight of adults from the north came through the area a generation earlier and laid eggs in the freshly cut alfalfa fields. Colias apparently migrate more under certain environmental conditions than under others (Hovanitz, '43d, Williams and Bishara, '29) and it is very reasonable to assume such a mass migration at one time but not another. Females normally migrate to cut fields for egg laying. Since the fields are not all cut at the same time in an area, the infestation will be spotty. Thus, places like Greenfield and Rosedale may get an infestation which arose from farther north and which do not belong to the area. Lamont may have gotten a smaller influx.

The localities north of Rosedale all have a frequency above 63%; Shafter is highest at 70%. The latter is statistically different from all the populations other than Greenfield.

The average for all the localities north of the Kern River (Rosedale and north) is statistically higher than the area south of the Kern River:

		N
North of Kern River	64.50% ± 0.79%	6,450
South of Kern River	57.65% ± 0.69%	5,237

It seems that the segregation of a small area like that south of the Kern River could not be maintained at lower gene frequency without either a complete isolation with no selection for any allele, or a selection rate for an allele which is at par with the influx of the other allele. There is no isolation except distance between the above two areas. The only reasonably close populations to this area are in the north; the gene frequencies there are all high. The butterflies are known to fly great distances and to move in great quantities. Therefore, there seems to be no alternative other than to assume selection against a very high concentration of dominant white alleles in the southern area. This selection for the area must eliminate as many dominant alleles per year as migrate into the region from the north. There is no higher migration of recessive alleles from any direction into the southern area.

The samples obtained from Rosedale and Base in September were very small, for cold weather had killed most of the brood indirectly (through action of parasites on the eggs and young larvae). The graph (fig.2) shows that the samples were of nearly the same frequency as in August; the slight increase was not significant:

			N
Rosedale	(September)	67.65% ± 2.88%	272
Base	(September)	55.56% ± 4.86%	188

Most of the butterflies were very old and worn at this time suggesting that these were stragglers from the August emergence. It is quite likely, for the September cool weather would not have allowed a new generation but would have allowed the adults to live longer. Eggs laid in August were noted dead on the alfalfa leaves in great quantities.

June and July averages for the South San Joaquin Valley area are as follows. These are hardly comparable, owing to the additional and different populations sampled in August but they are statistically different:

		N
June	57.06% ± 0.72%	4,932
August	60.50% - 0.46%	8,983

The North San Joaquin Valley Area: Samples were made in this area at distances averaging about the same as those in the South San Joaquin Valley area, namely, 6 to 10 miles. The area is depicted on the large map by a triangle (fig.1). The details of each sample are shown on the enlarged map (fig.5). The distance along the hypotenuse of the triangle is about 56 miles; each of the other sides are about 35 miles. Most of the localities sampled lie almost in a straight line along the hypotenuse; this compares with the longest length of the South San Joaquin Valley area. One locality lies at the right angle corner and each of the other two are away from it toward the acute angles. Separating the line of seven populations along the hypotenuse from the other three locations is the meandering San Joaquin River and its flood basin. Owing to this break in the valley agricultural area, the distances between the west San Joaquin River populations and the east San Joaquin Valley areas are nearly twice normal.

Alfalfa fields in this triangular area are most abundant along the zone between the San Joaquin River and the Coast Range hills. The hypotenuse of the triangle roughly designates the edge of the hills. The alfalfa fields are less abundant on the east side of the San Joaquin Valley and disappear beyond Ripon. There are few directly along the San Joaquin River. South of Newman and north of "County Line" the fields are still quite numerous. In the Coast Range hills, there are none for many miles.

A sample was made at Tracy on June 7, 1942. All the other samples including another at Tracy were made in the ~~four~~ two-day period, August 14 to 18, 1942. One population, Westley, was again sampled on the 18th. The frequency in June at this place was not very dif-

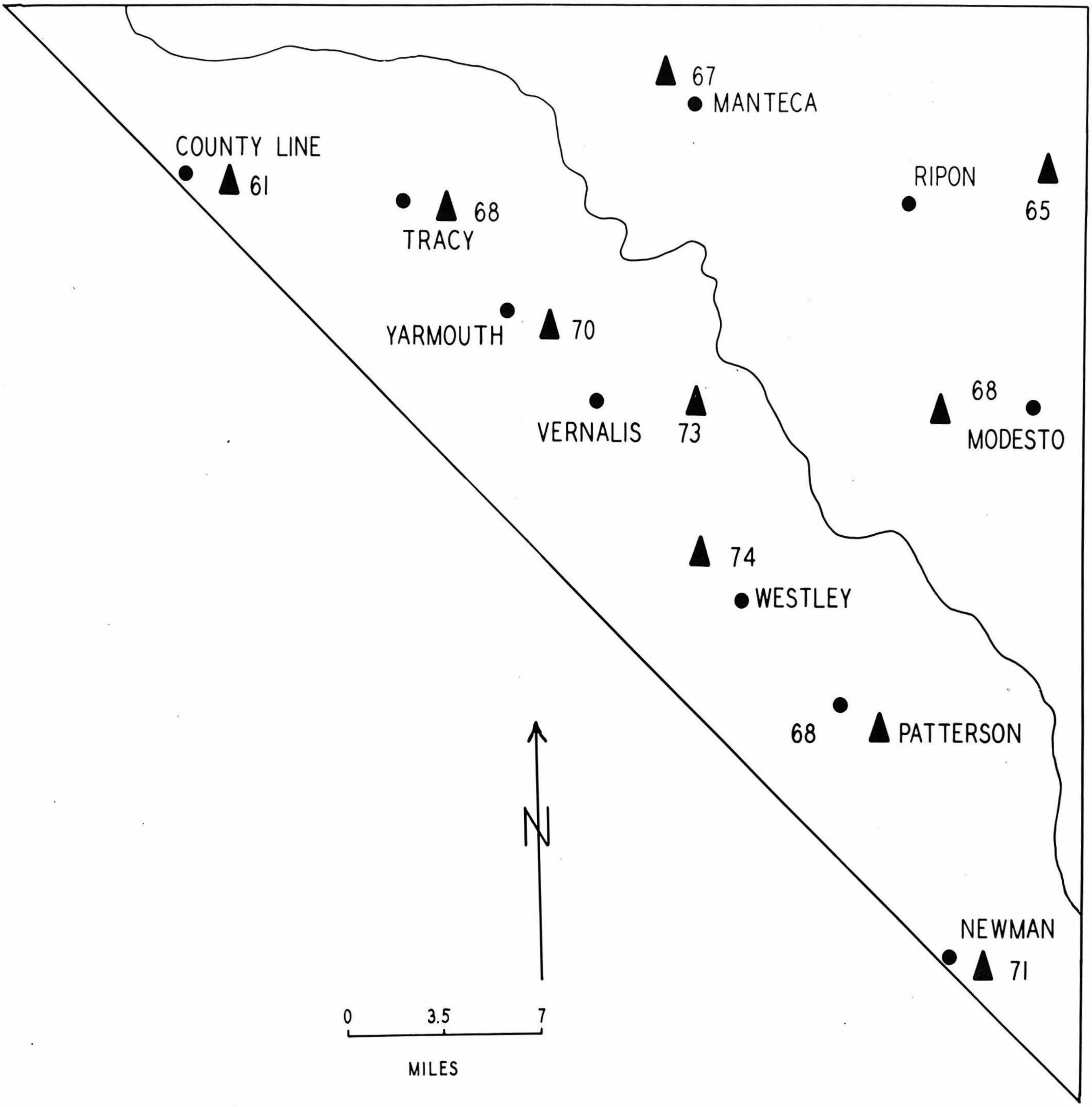

Fig. 5 .-- North San Joaquin Valley area represented on the map of California by a triangle. Waving line is the San Joaquin River. Small triangles are locations of alfalfa fields sampled and dots are places from which the names have been obtained.

ferent from that in August, the slight increase not being significant (fig.2):

Tracy		N
June	64.67% ± 2.73%	300
August	67.88% ± 1.36%	1,180

The August frequencies suggest a concentration of white genes in the region around Westley. From there to the east and north (and also possibly to the south) the frequency decreases. These differences are statistically significant. The averages for the three central populations, Westley, Vernalis and Yarmouth, are shown here for comparison with the two northwestern populations, the three eastern ones and the two southeastern ones:

	%white	N
Central (Westley, Vernalis, Yarmouth)	72.25% ± 0.86%	2,729
Northwest (Tracy, County Line)	64.85% ± 1.01%	2,202
East (Manteca, Ripon, Modesto)	66.77% ± 1.07%	1,950
Southeast (Patterson, Newman)	66.72% ± 1.55%	895

The central area is obviously different from each of the others except the southeast. The standard error of the difference of these two is just 2X the difference, e.g., 3.53% ± 1.78% Neither of the other three areas are different from one another, though some of the individual differences are great. "County Line" has a low of 61%, the lowest in the northern populations; it is statistically different from all the rest.

Judging from visible approximation, the population abundance was also highest in the central area, especially at Westley. At Yarmouth and Westley, the population consisted primarily of newly emerging adults; wet winged individuals were common. At the other localities, the individuals were apparently not so fresh. In a freshly cut alfalfa field, the ratio of males to females is a fair indication of the new emergence. A cut field with no new adults eclosing is nearly barren of males. Females in these places are laying eggs and are quite abundant. On the other hand, a freshly cut field with adults eclosing has a high frequency of males. The frequency of females which have wet wings and are in copulation is very high. Yarmouth and Westley were examples of the latter. The other fields except Tracy were all examples of the former. Tracy was rather intermediate.

At Westley, in view of the correlation between highest frequency of white females and greatest fresh eclosions, a sample of wet-winged (copulated) females was obtained. The following results, which did not seem reasonable at the time, was obtained:

		N
Westley, August 16, 1942		
Free-flying	73.92% ± 1.33%	1,112
Wet-winged (copulated)	60.84% ± 3.31%	166

It is clear that the frequency is very different. The frequency of white eclosions is lower than the frequency of the free-flying females. This is true ~~in spite of~~ despite the contamination of the free-flying population with individuals also eclosed on that day but already in flight. The tentative conclusion was drawn either that white females did not mate as freely with the orange ones (negative sexual selection) or that the brood had already mostly eclosed, with the whites eclosing first. It was for the purpose of testing these conclusions that the San Fernando Valley population already mentioned was analyzed in September - October, 1942 when it was observed that the conditions were fortunately excellent at that one place. Earlier, attempts had been made at getting frequencies of copulated females to compare with the general population frequency, this being an analysis of male sexual selection for the different colored females. Such attempts ended in failure because the females don't often mate after the original copulation at eclosion. A sample was obtained in a field of old females near Rosedale, June 25, 1942, with the following results:

		N
Free-flying	53.33% ± 1.31%	1,440
Copulated but not newly emerged	54.17% ± 7.18%	48

The number is small but quite the same in both cases. It is doubtful if the colored forms have any different sexual antipathy.

Two days after the sample described above was taken at Westley another sample was obtained. This time copulated females were more difficult to find. The suggestion is that the emergence was nearing its close. The results were:

Westley, August 18, 1942		N
Free-flying	71.12% ± 1.39%	1,056
Copulated (with wet wings)	65.38% ± 9.35%	26

The number of newly eclosed females is too low this time to have much significance. It is still lower than the general frequency. The frequency in the free-flying individuals has dropped 3% but the significance is doubtful (not quite 2X the standard error of the difference: 2.80% ± 1.64%).

If the white females had eclosed in a higher frequency earlier in the general population emergence, it would be expected that the white frequency in the general population would drop daily just as it did in the San Fernando Valley population. The above 3% drop may represent this difference.

Toward the end of the adult emergence it would be expected that the frequency of white females in the newly eclosing individuals would be lower than that in the general population. This, too, is true just as it was in the San Fernando Valley populations.

On the same grounds, it would be expected that the area where the adult emergence is taking place in greatest quantity would have the highest white frequency. This is true. The central area where the white female frequency is highest is also the area where the adults were emerging in the greatest quantity (especially at Yarmouth and Westley). From this central point of greatest abundance, they were probably flying in all directions. The high frequency at Vernalis where there was no emergence noticeable was due to the trap effect of the "isolated" alfalfa field where the sample was obtained. Being between Vernalis and Yarmouth, it would attract adults flying away from those centers.

These data, therefore, suggest that samples taken at different times of the brood emergence may not be quite typical of the general region.

The average frequency for the North San Joaquin Valley area is 68.50% ± 0.49% (N = 9,024). The percentage will be compared with others later.

<u>The Central San Joaquin Valley Area</u>: Four frequency samples were obtained at variously-spaced locations between the North and the South San Joaquin Valley areas. The valley is flat the entire distance but regions where alfalfa is grown are spotty. Therefore, some of the distance may be said to be a barrier to Colias movement by reason of space alone. The map (fig.1) illustrates the locations of the places. The two southernmost of these localities have the

lowest frequency but are not greatly different than would be expected, nor from what has been found in the more detailed zones. The 65.37% ± 11.29% (N = 891) Allensworth and the 68.42% ± 1.88% (N = 38) at Corcoran are close to the 64% average for the north end of the South San Joaquin Valley area. The Hanford 59.48 ± 8.50 ~~1.88%~~ (N=686) and the Madera 61.72% ± 1.69 (N = 802) are the lowest north of Bakersfield. The following explanation may be suggested for these figures:

(a) these may be just chance changes in the gene frequencies at various places owing to lack of climatic differences between the localities.

(b) alfalfa fields are not as common in this area and the populations are more isolated. Therefore, the population size (abundance) may be smaller allowing chance fluctuations greater leeway.

(c) the smaller population size may be sufficient to eliminate part of the selective advantage the white allele may have.

The Central San Joaquin Valley area as sampled by these four locations has a white female frequency lower than the north end of the South San Joaquin Valley, 62.52 versus 64.50 which is just over 2X the standard error of the difference. However, considering the South San Joaquin Valley as a whole, it is higher:

		N
North San Joaquin Valley	68.50% ± 0.48%	9,324
Central San Joaquin Valley	62.52% ± 0.97%	2,420
South San Joaquin Valley	59.39% ± 0.41%	14,375
TOTAL San Joaquin Valley	62.94% ± 0.29%	26,119

A rise in white female frequency is apparent from south to the north in the valley.

The San Francisco Bay Area: Alfalfa is apparently grown commonly only at the southeastern portion of the Bay Valley. Hence, the sample was obtained there (fig.1). Actually, several small areas were investigated but only the total value is of reasonable size. The average frequency here of 66.04% ± 3.70% (N = 159) is in agreement with the 68.50% ± o.48% (N ± 9,324) average for the San Joaquin Valley at the same latitude.

A summary of the frequencies in four of the five provinces in California is given in Table 6. The fifth province, the Great Basin, is not comparable and will be discussed below.

The Great Basin Area

The Great Basin Area was sampled in two locations, Mono Lake Valley, Mono County and Round Valley, Inyo County (map, fig.1).

The Mono Lake area: Mono Lake is situated about 180 miles airline directly east of San Francisco (fig.1). It is a Basin lake at an elevation of 6,300 feet above sea level and has no outlet. The country around it is very high. The Sierra Nevada mountain range is a continuous wall on the west with peaks up to 13,000 feet in elevation, with no passes lower than 10,000 feet. Mountains 8,000 to 10,000 feet high surround the basin on the other three sides. The country is very arid due to the rain-shadow effect of the western mountain range. Winters are exceedingly cold, going well below 0°F., and snow falls for several months of the year if there is any precipitation at all. Summers are often exceedingly hot but sometimes may be cold all year. The air is always dry.

Meadows watered by springs and melting snow in the mountains occur in several places around the western edge of the lake. In

Table 6.- Summary of the white female frequencies in the orange race *Colias chrysotheme* populations in California west of the Sierra Nevada but including the southwestern desert areas.

Locality	% white females σ	N
1. San Francisco Bay	66.04 ± 3.70	159
2. San Joaquin Valley		
North	68.50 ± 0.48	9,324
Central	62.52 ± 0.97	2,420
South	59.39 ± 0.41	14,375
3. Coastal Southern California	29.86 ± 0.34	18,617
4. Coachella-Imperial-Colorado River Valley area	16.31 ± 0.82	2,035
Grand Total	47.77 ± 0.23	47,010

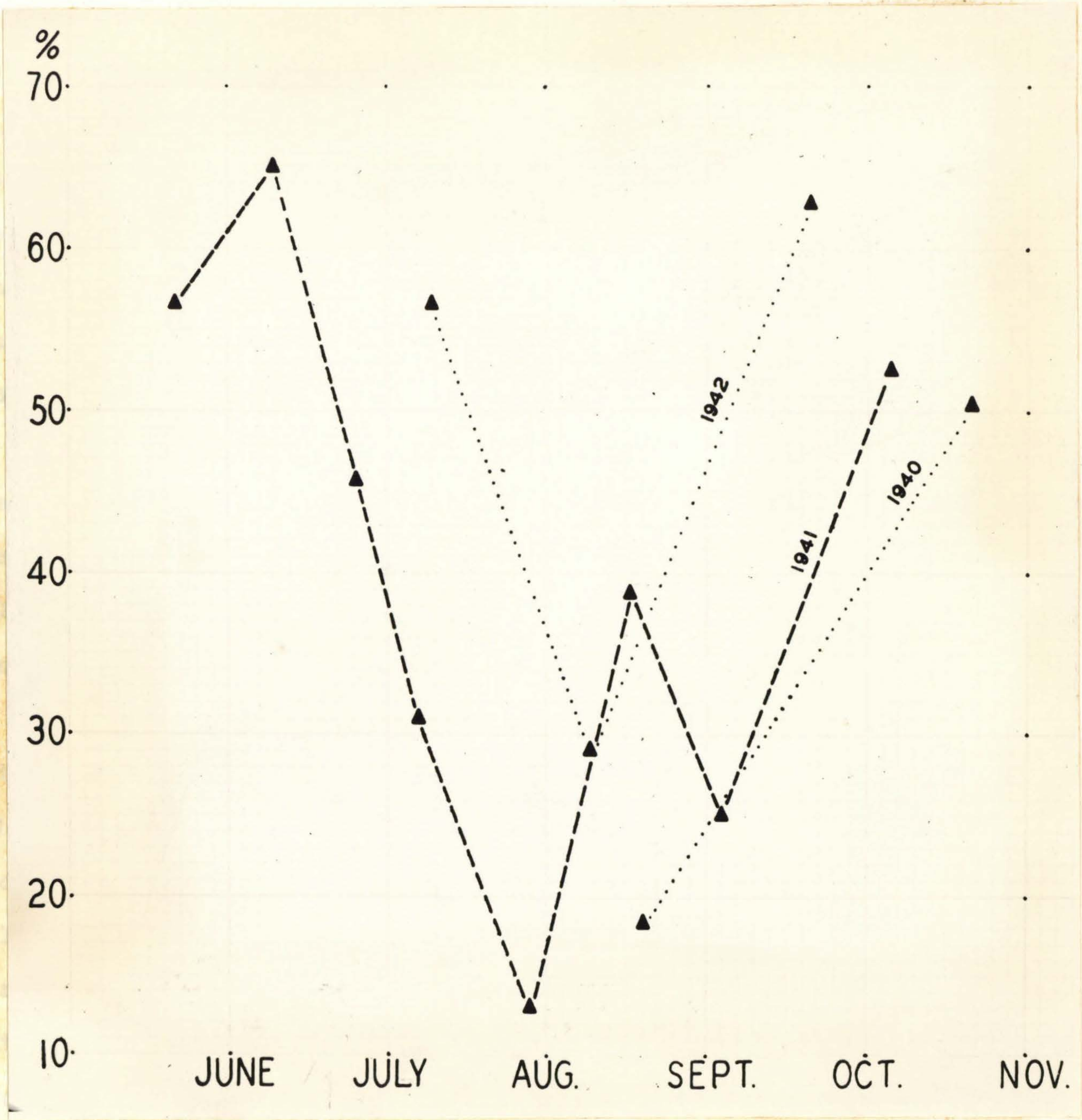

Fig. 6 .- Frequencies of white females during the season at Mono Lake, California. Note the great seasonal change and compare with fig. .

these meadows, native clovers of various sorts grow, as well as some alfalfa mixed with red and white clover planted by the native residents. The agriculture of the region is primitive; the fields or meadows are grazed by sheep or cattle rather than being cut for hay as in the western valleys. In the meadows, one finds the yellow race Colias at certain times of the year and nearly everywhere one finds the orange race. In the wet meadows both the yellow and the orange race seem to concentrate in the largest numbers and it is in two of these meadows that the frequencies of the white, yellow and orange forms were obtained. The yellow form presents an entirely different problem from the white form and will be dealt with separately (Hovanitz '43c).

The seasonal fluctuations in the white female frequency were great. Early in the season for that place the frequency was high; in the middle of the summer it was low. Again in the fall, the frequency rose again to nearly its early height. This change in seasonal frequency is illustrated on the graph (fig.6) for the three years, 1940, 1941 and 1942. The fundamental change is the same in all three years. The major portions of the samples making up the curves are statistically significant (Table 7). At times it was very difficult to get a reasonable sample and at first (until the middle of 1941) all samples were obtained by capturing the females with a net,

By way of summary, the 1941 season started out in May and early June at 55-65% white females, then dropped through 45% in late June, 30% the first of July to a low of 15% at the end of July. This rose gradually through August and September to a high again of

Table 7.- The frequency of white females throughout the season at Mono Lake, Calif. This is the frequency as compared with the normal orange females of the orange race.

Date	%white females	σ	N
1940			
Aug. 11	18.2 ± 11.1		11
Oct. 20	50.0 ± 8.48		34
1941			
May 19	56.82 ± 7.45		44
June 8	64.96 ± 4.36		117
June 24	45.75 ± 4.04		153
July 5	31.08 ± 2.22		415
July 26	13.13 ± 3.41		99
Aug. 15	38.74 ± 4.56		111
Sept. 2	25.0		4
Oct. 4	52.27 ± 7.45		44
1942			
July 7	56.30 ± 3.21		238
Aug. 7	29.23 ± 2.82		260
Sept. 16	62.89 ± 3.46		194

52% in early October.

In 1942, the season started late at Mono Lake for the orange race. In early July, the frequency was at about 58% white. By the first of August this had dropped to at least 29% and rose again in late September to a high of 62%. The samples were not made at as frequent an interval during 1942 as in 1941; hence, the actual low point might have been missed. The 1940 collections are small but, nevertheless, follow the trend perfectly.

This great seasonal change in white female frequency is not duplicated in any of the populations considered heretofore (fig.2). It is not an effect of the direct influence of the environment upon the phenotype of the adult for the character is completely under genetic control (Hovanitz '43a).

A gene frequency alteration of the proportions needed to change the percentage of white females from 65% to 13% at Mono Lake is the same as would be necessary to change the population in the North San Joaquin Valley to equal those in the Imperial Valley. This change takes place in as short a period of time as 1½ months. For the Mono Lake area, it takes place in time equal to the developing period for one generation.

Several possibilities to account for this rapid gene frequency change have been considered:

(a) That there is a tremendous environmental selection in the area against the recessive orange allele in winter due to the very cold winters and in favor of the recessive allele in summer due to the hot, dry weather. The selection during the winter is easy enough to imagine but the summer change is so rapid that it does not seem possible. Even with the aid of a possible homozygous lethal dominant, which would change the frequency from 66.7% to 50% in one generation, it is too great a change to expect. The climatic change is not that abrupt or extreme.

(b) That the homozygous dominant white female (and male) is lethal in warmer weather. This would reduce the population very rapidly from 66.7% to 50% to 28.6% to 25%, etc. per generation or in terms of gene frequencies from 33.3% to 25% to 14.3% etc. (Table 1). It would take more than 9 generations to get from 65% to 13% at this rate and only one is possible.

(c) That white females are at a predatory disadvantage and are likely to be eliminated in the summer more than at the colder parts of the year. The degree of bird attacks on adult butterflies of Colias was closely and carefully observed during the sampling of all the populations in 1941 and 1942. It must be considered that predatory attacks are negligible, though not completely absent.

(d) That there is an alternation of generations during the season such that spring and autumn individuals represent a really different population from the midsummer one. In some of its aspects, this is the conclusion accepted. However, it is not believed that both "populations" live and breed at Mono Lake. If they did there would have to be almost no intermixing in order to prevent gene interchange. No break of this sort is observed at Mono Lake in the orange race. Instead there is a population "high" in midsummer and decrease toward spring and fall , rather than discrete broods.

(e) That the individuals at Mono Lake have migrated there from some other place so that at certain times of the year the individuals are representative of some other locality. This would really be an alternation of "generations" at the locality. At first, this possibility seemed impossible but later evidence has made it seem very likely. The reasons are as follows:

I The exceptionally rapid gene-frequency alteration is most reasonable on these grounds. The presence of individuals from a high-frequency population at one time of the year and from a low-frequency population at another time is the easiest explanation.

II Populations of high frequency (60 to 70%) exist just west of the Sierra Nevada from where migrations could have had their origin (San Joaquin Valley). The Great Basin and Mountain Region have a low dominant gene frequency (14%) as judged by museum data (Hovanitz '43b). Nevada and eastern California have frequencies of 19% and 17% respectively, (N = 43 and 65). Much of the latter material is probably yellow race since there is only 29% orange race calculated in the above figures. Apparently the environmental conditions in the dry mountain and basin region are most favorable for the lower dominant allele frequency. It is not believed that there is any migration of individuals into the Mono Lake area from low-frequency areas but rather that the low-frequency individuals represent the resident population.

III When the white frequency is high, the individuals are invariably old and worn. When low, the individuals are much fresher and newly eclosed as if they had been bred in the vicinity.

IV At the times of high frequency early and late in the season the adults flying are of a phenotype development which could not have occurred in the cold weather of the Mono Lake climate. Were the pupae or larvae exposed to the cold night temperatures of the vicinity, they would be reduced forms comparable to "spring forms" in the low lands. A very few "spring form" individuals do occur early in the season and especially in the autumn. These are the resident individuals. Most adults at those times are worn "summer form" and were probably grown at a lower elevation. Later, as the resident population increases in size, it ~~makes~~ masks the high white frequency of the immigrant population. This would account for the gradual rise in the recessive orange allele frequency with a lack off a definite series of broods during the season as is found in the San Joaquin Valley (Michelbacher and Smith, M.S.). It was originally assumed that the adults found at Mono Lake in May were overwintering individuals because of the "summer" phenotype development they possessed. Such a supposition had been made as long ago as the nineteenth century by Mead and Edwards (Edwards, '63-'98). They found that adults high in the Colorado Rockies early in the season were also "summer" individuals. The high improbability that Colias adults can overwinter in the true sense is shown by the length of time they can be kept in a cold room at extremely high humidities and normal low humidities. At 2° C., females will live inactive for 3 to 4 weeks or possibly a little longer at an extremely high humidity. At a normal low humidity they will die in 1 to 2 weeks at that temperature. The dryness of the Mono Lake atmosphere would kill the butterflies there quickly if the cold did not.

That Colias of the orange race can fly distances as great as the 100 miles from the San Joaquin Valley to Mono Lake is illustrated by the many migratory individuals taken in the far northern parts of North America (Hudson Bay, Northern Manitoba, Alberta, etc..) where overwintering populations do not exist. "Summer-form" individuals of the orange race have been taken in the New England states very early in the season. They must have flown in from the South.

The ability to travel is great in the orange race of *Colias chrysotheme* (Hovanitz '43d). Summer form females have been observed laying eggs high in the San Jacinto mountains of Southern California in May and June where temperatures in the day are cool and at night near freezing. They could not have overwintered there but must have come from the alfalfa fields in the valleys below.

The orange race is fairly abundant in the Hudsonian and Alpine life-zones of the Sierra Nevada in Midsummer. It is exceedingly doubtful that they could have overwintered in such a cold place but it is more likely that they have been reintroduced into the mountains seasonally. Apparently, a reintroduction takes place in the north such as in Alberta every few years (Bowman '42). *Colias behri* is the resident Colias of the Hudsonian meadows in the Sierra Nevada, to which place it is restricted.

The question of why the Mono Lake population never reaches equilibrium with the San Joaquin populations probably involves several factors. The resident individuals may be better able to withstand the cold winters just as the orange race in the north-eastern United States can now better withstand the winters there. Or, the low frequency of the populations throughout the Basin and Mountain area may just absorb the excess white genes coming from the valley. Climatic selection in the area may be sufficient to reduce the frequency after a period of time. Or, the low white allele frequency in the yellow race of the area may absorb and eliminate many of the genes through intercrossing. The yellow race is apparently better adapted to resident life in this region judging from its frequency of 71% for the mountain area in the museum data. Or, the dominant gene may have a disadvantage in the genom typical of the Basin region which would render the homozygote-dominant lethal or reduce the white heterozygote frequency.

The average frequency of white females at Mono Lake is 43.16% ± 1.18% (N = 1,724).

<u>The Round Valley area</u>: Round Valley is south of Mono Lake 60 miles. It is part of Owens Valley and is 2,000 feet lower than Mono Lake, or 4,500 feet. The winters are not quite so cold as Mono Lake and the summers are very much hotter. The warm season is several months longer. The general relationship to the Sierra Nevada range and San Joaquin Valley is the same as Mono Lake. There are, however, more alfalfa fields in the vicinity, especially to the south in Owens Valley. Round Valley itself is a big meadow with mixed native clovers, white clover, red clover, alfalfa and grass fields kept green by irrigation and springs.

The frequencies of white females obtained at this locality ~~was~~ were purely incidental to the study of the orange-yellow relationships. Therefore, the numbers in the samples are small (Table 8). Apparently, however, the frequency may not fluctuate seasonally as as Mono Lake since in July of 1942 a frequency of 58% was procured. This may be accounted for by the more desert environment around Round Valley such that the orange race population is completely restricted to alfalfa fields, Apparently, there is a great migration of Colias over the Sierra Nevada to Round Valley just as to Mono Lake. Without a native population of orange race other than that one in alfalfa fields, the high white frequency is not reduced much by swamping of the genes.

The average of white females during 1941 and 1942 was 53.61% ± 3.04% (N = ~~3.04~~ 263). This is statistically higher than Mono Lake's

Table 8.- The freqgency of white females throughout the season at Round Valley, Calif. This is the frequency as compared with the normal orange females of the orange race. However, in 1942 66% of the females other than white at this place were of the yellow race.

Date	% white females σ	N
1941, season from May-Oct.	35.13 ± 7.84	37
1942		
April 1	28.6	7
April 25	50.0 ± 10.2	24
June 12	53.85 ± 9.78	26
July 8	58.46 ± 4.32	130
Aug. 6	65.22	8
Sept. 16	51.61 ± 8.98	31
tatal	56.64 ± 3.42	226
1941-42 total	53.61 ± 3.04	263

43.16% ± 1.18% (N = ~~4~~1,724). The north-south rule is reversed and the climatic expectancies are also reversed. However, possible reasons for this condition have already been covered. (Probable lack of resident population). The frequency of the total for Mono Lake and Round Valley is 44.53% ± 1.06% (N = 1,987). This total is much higher than the 17% determined from museum material for the eastern Sierra Nevada but the latter was based on 70% yellow race contamination. White females in the yellow race at Mono Lake and Round Valley are either totally absent or close to it.

Summary and Conclusions

1. The frequency distribution of white females in the orange race of Colias chrysotheme (= eurytheme) has been shown to change from 74% in the North San Joaquin Valley to 13% in the Imperial Valley. This change in frequency has been shown to be largely correlated with the change in climatic conditions through the area.

2. The greatest change in frequency between populations takes place where there is complete isolation between populations as well as climatic differences. However, an environmental selection for the alleles controlling the characters is shown to be present even when no isolation exists between populations (San Joaquin Valley).

3. Wild population changes in gene frequency of various magnitudes have been illustrated. Most of these are shown to be due to population movement and others to a differential development rate of the genotypes.

4. The causes of population differences have been shown to lie in any of the following: (a) possible random deviations, (b) climatic selection, (c) differential population movement and migration, and (d) different age of the population after eclosion.

5. The faster development rate of white females (heterozygous plus homozygous dominant) over the orange (homozygous recessive) is assumed from some of the population analyses.

6. No differential sexual selection by the males for the polymorphic females is noted.

7. The calculation of actual gene frequencies from wild population phenotype frequencies in any organism can never be accurate so long as knowledge of the viability of the three genotypes ($p^2 + 2pg + q^2$) under known environmental conditions can not be controlled, only good estimates can be made of the true gene frequency.

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