words PB to some extent still affects the cells by the end of the experiment and so the delay of the CHX-related lag phase is not the result of a simple decay, inactivation or dilution of PB. In this sense PB is not a simple antagonistic factor against CHX, but a factor which modifies the whole temporal sequence of the response to CHX.

Therefore another model is proposed; PB pretreatment of *Chilodonella* cells stimulates some short-lasting and gradually-disappearing effects, preventing temporarily an inhibitory action of CHX, but it also enhances the cellular activity needed for a more rapid recovery from CHX action. If PB pretreatment temporarily induces an action of enzymes to inactivate most of the CHX molecules, or prevents their activity against the cell, then the concentration of the active CHX molecules at the start of the experiment is too low to inhibit the protein synthesis and to stop cell divisions. However, this pretreatment does not prevent a gradual accumulation of active CHX molecules during the next 2-3 days of CHX treatment. When the critical concentration of CHX molecules is reached within the cells, they fall into the 'lag phase'. But there are still other effects of PB pretreatment which make cells more ready to adapt to CHX. Thus the period of recovery from the 'lag phase' to the control level of the fission rate is shortened.

Based upon known caracteristics of barbitals and upon experiments done on liver cells[13,20-25], one can suggest that an activation of some drug-inactivating system or/and an increased level of transcription and translation induced by PB might be involved in the specific temporal pattern of reactions of *Chilodonella* to CHX following PB pretreatment. While this explanation seems plausible, there is no direct evidence regarding the pathways sensitive to PB in ciliates, apart from the finding of cytochrom b_5 in Tetrahymena[26], which is involved in such a pathway in liver cells.

1 Thanks are due to Dr J. Kaczanowska and Dr A. Kaczanowski for instructive discussions, to Prof. Dr G. de Haller and Dr D. Wheatley for critical reading of the manuscript.
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Enzyme electrophoretic approach to the systematics and evolution of the butterfly *Euchloe ausonia*[1]

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Summary. Enzyme electrophoretic studies show that the *Euchloe* taxa *simplonia* and *crameri* share no common gene pool and are genetically as much differentiated from each other as from the North American *E. olympia.* It is concluded that the taxa *simplonia* and *crameri* are specifically distinct; separation of their gene pool must have occurred long before the end of the last glacial period.

Butterflies of the pierid genus *Euchloe* Hbn. (Lepidoptera, Pieridae) are distributed over the holarctic region. The taxa of this genus show a remarkable uniformity in coloration and wing pattern which often makes their identification rather difficult. Among the European members of this genus, *Euchloe ausonia* is one of these problematic taxa: a great variety of subspecies, races or forms has been described whose systematic rank is still unclear. In the most recent revision, Back[2] referred to *E. ausonia* from the European continent and North Africa as a complex which he subdivided into 3 groups: a) '*crameri*-group' (range of distribution: northwest Africa, Spain, France and Liguria eastward to Genoa); b) '*ausonia*-group' (Italy, from Modena south- and eastward, to Asia Minor); c) '*simplonia*-group' (Alps and Pyrenees). However, the systematic relationships among these 3 groups remain unclear, in particular since data on genetic isolation are not yet available.

A convenient approach for investigating genetic relationship among populations is provided by enzyme electrophoretic analysis[3]. Populations sharing a common gene pool will in general be very similar in allelic compositions at individual enzyme loci. However, following an interruption of gene flow, the populations tend to diverge and accumulate different alleles. In general, the degree of genetic differentiation depends mainly on the period of time since the separation from a common gene pool[4]. In fact, bio-

chemically detectable genetic divergence has been demonstrated by various investigators[5] to be closely correlated with the phylogenetic age of the taxa involved.

The electrophoretic studies presented in this paper were therefore initiated to give answers to the following questions: 1. Is it possible to distinguish taxa within the European *'ausonia'* complex on the basis of enzyme electrophoretic analysis? 2. What ist the degree of genetic differentiation between such taxa and in comparison with well defined species of the genus *Euchloe?* 3. What are the relative ages of the taxa investigated?

Material and methods. Butterflies were collected from the following sites (number of butterflies in brackets):

a) *'ausonia'* complex[2], *simplonia*-group: Switzerland: Ausserberg, VS (14), Grindelwald, BE (5). France: Font Romeu, Pyr. Or. (7). *Crameri*-group: France: Orgnac l'Aven, Ard. (13), Banyuls-sur-mer, Pyr. Or. (3), Bollène, Vaucl. (12), Oppidum d'Enserune, Hér. (7). Spain: Alicante (1). Italy: Modena (11).

b) *Euchloe olympia* Edw.: Canada, Ontario (7).

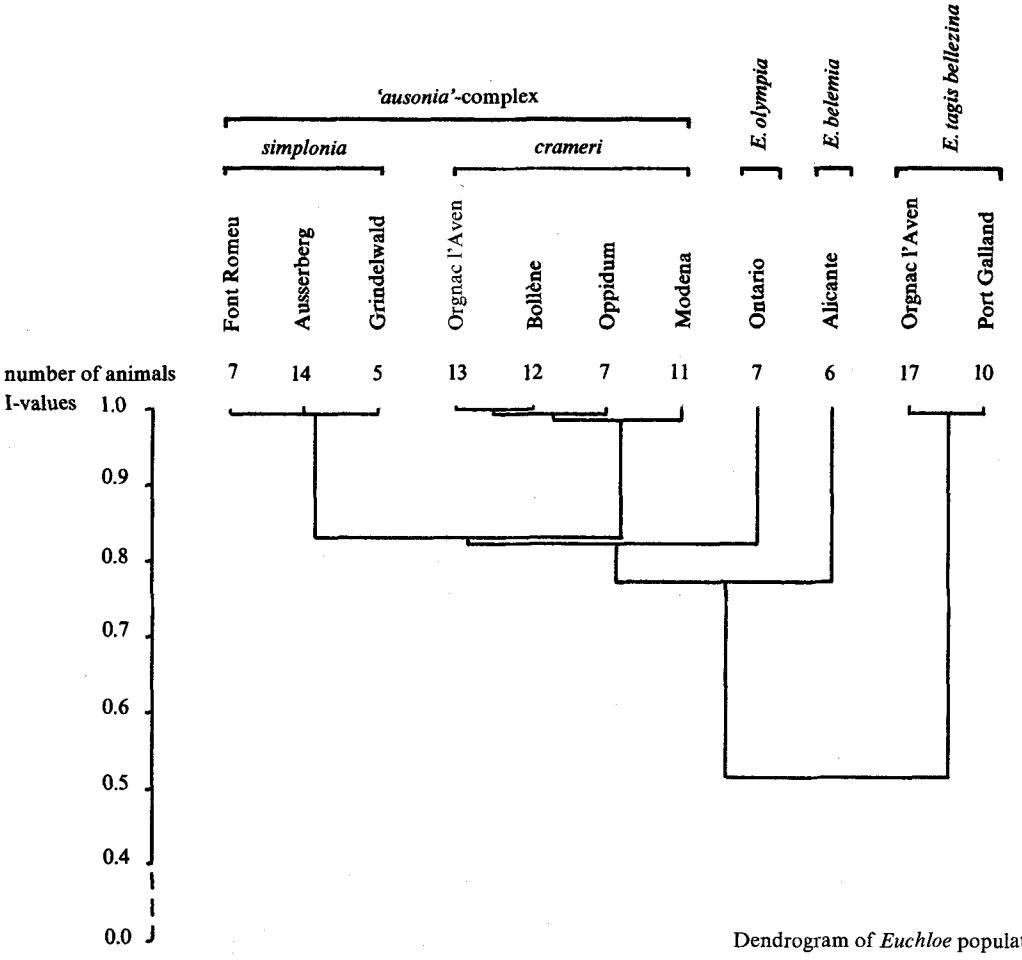
c) *Euchloe belemia belemia* Esp.: Spain, Alicante (6).

d) *Euchloe tagis bellezina* Bois.: France, Orgnac l'Aven, Ard. (17), Port Galland, Ain (10).

The thoraxes of freshly killed, deep-frozen (−35 °C) or lyophilized butterflies were homogenized in 10 volumes 0.1 M Tris-HCl buffer (pH 8.0) and centrifuged at 18,000 × g. Supernatant fractions were used for vertical starch gel electrophoresis following standard procedures for electrophoretic separation and enzyme staining[6,7]. 21 enzyme loci have been scored (number of loci in brackets): adenylate kinase (2), glyceraldehydephosphate dehydrogenase, arginine kinase, malate dehydrogenase (2), aldolase, indophenol oxydase, malic enzyme, hexokinase, pyruvate kinase, glutamate-oxaloacetate transaminase (GOT-1 and GOT-2), 6-phosphogluconate dehydrogenase (6-PGD), fumarase, isocitrate dehydrogenase (2), glutamate pyruvate transaminase, phosphoglucomutase (PGM), phosphoglucose isomerase, α-glycerophosphate dehydrogenase. The statistical method of I was used to estimate the degree of genetic similarity between the samples studied[8]. A dendrogram (fig.) was generated using the UPGMA-method[9].

Results. In regard to the *'ausonia'*-complex, 2 groups of populations may be recognized. Within these groups only minor differences in gene frequencies are apparent. However, between these groups a remarkable degree of genetic differentiation is observed. In particular, no common alleles are detectable at the PGM-, GOT-1 and 6-PGD-locus. Group 1 comprises the samples from the Swiss Alps (Ausserberg and Grindelwald) and the Pyrenees (Font Romeu); group 2 comprises the samples from the populations of Orgnac l'Aven, Banyuls-sur-mer, Bollène and Oppidum d'Enserune (France) and Modena (Italy) as well as a single animal from Alicante (Spain). In fact, group 1 is represented by the taxon *simplonia* and group 2 by the taxon *crameri.* The 2 groups cluster at approximately the same level of genetic identity as the North American species *E. olympia* and the Spanish *E. belemia,* whereas *E. tagis bellezina* is separated by another clear step of lower genetic identity (fig.).

Discussion. The biochemical genetic data obtained from this study support the opinion of previous investigators[2] that the European *'ausonia'*-complex consists of distinct



Dendrogram of *Euchloe* population samples

groups. Our data provide strong evidence for regarding *simplonia* and *crameri* as specifically distinct for the following reasons: a) Between populations of the same taxon only minor differences in gene frequencies are detectable; the coefficients of genetic similarity (fig.), therefore, are always close to 1.00. b) Populations of different taxa, however, differ both quantitatively (in gene frequencies at polymorphic loci) and qualitatively (gene substitutions, in our material at 3 out of 21 loci investigated), which results in a considerably lower coefficient of genetic identity. This is a strong argument indicating that the taxa *simplonia* and *crameri* do not share a common gene pool. c) The genetic differentiation observed between North American species, *E. olympia*, and the European *'ausonia'*-complex is almost the same as that observed between *crameri* and *simplonia*.

With regard to the evolution of the genus *Euchloe* it is interesting to note that the taxa *simplonia* and *crameri* branch at the same level of genetic identity as the North American species *E. olympia*. Following the view that the electrophoretically detectable degree of genetic differentiation mainly depends on the periods of time since separation from a common ancestor, this would indicate that these 3 taxa evolved approximately at the same time. This event must have occurred after faunal exchange between Eurasia and North America was interrupted, but long before the gene flow between the *simplonia* populations, now restricted to the Alps and Pyrenees, was terminated (fig.).

The Bering Strait is often considered as a pathway of exchange between North American and Eurasian animals. This connection broke down during the last glacial period, i.e. 13,000–15,000 years ago. A period of 10,000 years, however, would not account for the rather high degree of genetic differentiation observed between the taxa *simplonia* and *crameri*, because the *simplonia* populations from the Alps and Pyrenees (reproductively isolated at least since the end of the last glacial period) have only reached a very low degree of genetic differentiation. This indicates that the taxa *simplonia, crameri* and *olympia* were probably separated much earlier. The time of their radiation may even be dated back to the tertiary period, when the Thule land-bridge, another connection between Eurasia and North America, was present. According to Friedrich and Simonarson[10], a last contact via this land-bridge probably existed 2 million years ago.

1 Acknowledgments. We are indepted to Mr J.H. Robert, Drs F. Llorca and A. Manjon, Alicante, Spain and Mr J. Reichel, Revelstoke, Canada, for material used in this study. Mrs V. Siegfried and Mrs L. Frauchiger, Berne, assisted in the electrophoretic investigations, Prof. H.P. Bulnheim, Hamburg, Germany, read the english version of the manuscript. This study was supported by the Swiss National Science Foundation grant No. 3.640.80.

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Morphological effects of the flavone isovitexin in a non-glycosylating genotype of *Silene pratensis* (Caryophyllaceae)

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Summary. Genetic studies have shown that the unglycosylated flavone isovitexin causes an aberrant petal morphology in *Silene pratensis*. Scanning electron micrographs show that the individuals with free isovitexin have abnormal upper epidermal cells.

The primary biochemical effect of a morphological mutant is only known in a few cases in *Drosophila*[2–5], although morphological and biochemical mutants have been identified for many organisms. In this paper we report on a genotype of *Silene pratensis* (= *S. alba*) with abnormal flower morphology apparently caused by the presence of the flavone isovitexin in the free, unglycosylated state. Plants with free isovitexin in the petals can be synthesized by genetic techniques, but also occur in nature as recombinants. All such plants show a characteristic abnormality in petal morphology. Incrossing of a dominant isovitexin glycosylation gene abolishes this effect. We shall show that the abnormal petals are caused by the presence of isovitexin in the upper epidermis of the petals, resulting in an aberrant cell morphology and premature cell death.

Flavonoids are universally present in the plant kingdom and show a bewildering amount of variation. Their significance remains a mystery, partly because their diversity makes it difficult to identify specific processes which they might control. The possibility that the flavonoids present in a given species are relicts of earlier adaptation processes cannot generally be excluded. Studies aiming to discover the functions of flavonoids should therefore be concentrated on species in which there are reasons to believe that the flavonoid spectrum has evolved fairly recently. It is also important that mutants of the flavonoid spectrum be available, so that the effects of variation in flavonoid composition on plant development can be investigated, and that the biochemical pathways affected in these mutants should be thoroughly known.

All these criteria are met in *Silene pratensis* and *S. dioica*, 2 species of differing flavonoid composition[6] which are thought to have had a relatively recent common ancestor. The biosynthesis and genetics of all the flavonoids shown to be present in these species have been elucidated. The use of genetic techniques allows the synthesis of plants with any