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.

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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

given flavonoid composition. It has been shown that flavone variation in the petals of *S. pratensis* is controlled by at least 3 independently-segregating loci, g, gl, and fg, with 3, 3 and 2 alleles respectively[7]. All these loci are involved in the glycosylation of isovitexin, the only basic flavone present in the petals. The dominant alleles g*G and g*X at the g-locus take care of the binding of glucose[8] and xylose[9] respectively to the 7-OH group of isovitexin. The other 2 loci control glycosylation of the 2″-OH group on the glucose part of the isovitexin molecule[10]. Glucose is bound to this group, when the dominant allele Fg is present. The 2 dominant alleles, gl*A and gl*R at the gl locus control the binding of arabinose[10] and rhamnose respectively to this same 2″-OH group (fig. 1).

Biochemical studies have shown that these dominant alleles are the structural genes for the respective isovitexin: UDP-glycosyltransferases[8-11]. The alleles g, gl and fg are recessive and do not code for an active isovitexin UDP-glycosyltransferase. Individuals in which the recessive alleles are homozygous at all 3 loci have a pronounced abnormality in petal morphology. The petals of such plants are smaller than normal and curl up easily (fig. 2a and b). This abnormality is removed by the introduction of any of the isovitexin glycosylation genes (fig. 2c and d). Since there cannot be a coding gene for this aberrant morphology which is linked to all 3 independently segregating loci, we infer that the

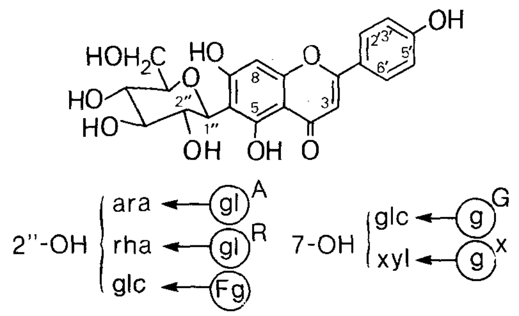

Figure 1. Genetic control of isovitexin glycosylation in the petals of *Silene pratensis*. The binding of glucose or xylose to the 7-OH group is governed by the alleles g*G and g*X respectively. The genes gl*A, gl*R and Fg control the binding of arabinose, rhamnose und glucose respectively to the 2″-OH group of the carbon-carbon bound glucose.

Figure 2. Morphological effects of the non-O-glycosylated flavone isovitexin on the petal structure. In individuals of the non-glycosylating genotype the petals are smaller and curl up more easily (a and b). This effect is removed by the introduction of a dominant isovitexin glycosylation gene (c and d).

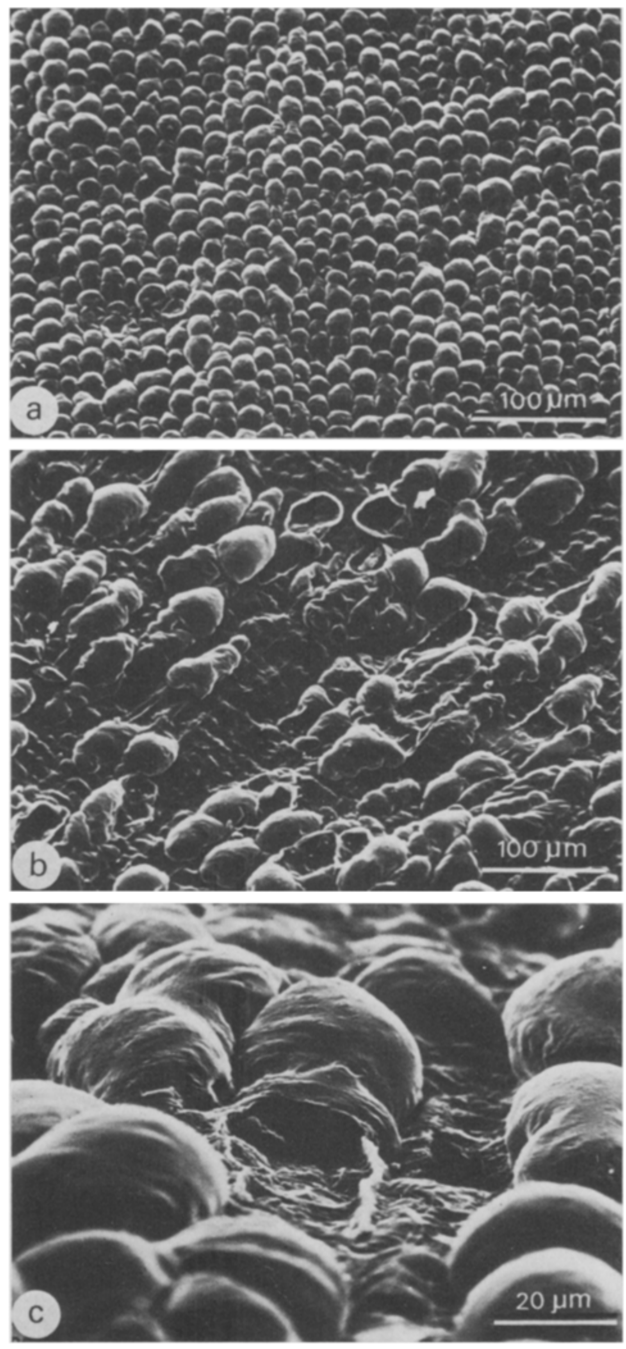

Figure 3. Scanning electron micrographs of the upper epidermis of *Silene pratensis* petals. In lines in which an O-glycosylated derivative of isovitexin is present, there is a regular structure of normally developed cells (a). *b* and *c* show the structure and arrangement of the epidermal cells in individuals of the non-glycosylating genotype.

non-O-glycosylated basic flavone isovitexin itself causes this striking morphological effect.

A microscope with quartz optics in combination with a monochromator enabled us to locate flavones. These studies showed that the isovitexin (glycosides) are mainly present in the vacuoles of the upper epidermis cells (unpublished results). Scanning elctron microscopic studies of petal surfaces from individuals with and without a glycosylated derivative of isovitexin revealed little difference in the lower epidermis; in both cases the elongated cells fit as in a jigsaw puzzle and are arranged in regular arrays. The upper epidermis of petals from normal plants also shows a regular appearance and arrangement (fig. 3a), but the cells in the upper epidermis of petals of the aberrant plants we found to be much less regular. Swollen cells are succeeded by groups of flat cells, and some of the swollen cells appear to have burst, their rims still protruding (fig. 3b and c). The different types of aberrant cells are not evenly distributed over the upper epidermis. In the basal part of the petal swollen cells are the most common cell type; burst cells are most common in the central part. Flat cells are especially numerous towards the apex.

It appears then that isovitexin has some toxic effect on the cells of the upper epidermis. This toxication leads to swelling of the cells at the base of the petal; in the middle of the petal it leads to collapse, whereas the flat cells at the top either are the debris of the burst cells, or are cells which have failed to develop.

The question remains why in *Silene pratensis* the accumulation of the flavone isovitexin in the vacuole causes this morphological effect. It is possible that the glycosylation of isovitexin prevents leakage across the tonoplast and thus prevents its interaction with the cell components of the cytoplasm. In vitro, flavonoids can influence many processes at very low concentrations, varying from indole acetic acid catabolism and hence hormone balance[12-14], to DNA replication[15-17] and oxidative- and photo-phosphorylation[14,18,19,21]. Among others Stenlid[14] showed that flavones can act as potent inhibitors of ATP synthesis in mitochondria, comparable to the classical uncoupler dinitrophenol. Flavone aglycones are in this respect many times more active than their glycosylated derivatives[13,14,18]. We hypothesize that free isovitexin interferes with the energy supply of the upper epidermal cells, which therefore have difficulties with the maintenance of turgor, leading to swelling and ultimately to bursting. The protruding debris of these cells may be rubbed off and thus they give the impression of flat cells. Finally, the damage done to the upper epidermis leads to the curling-up of the petals.

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Effect in heavy meromyosin on conformation of F-actin

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Summary. Cooperative conformational changes of F-actin induced by heavy meromyosin (HMM) binding (in the absence of troponin and tropomyosin) were found by the method of polarized UV-fluorescence microscopy.

Binding of myosin to F-actin is known to be an important moment in the generation of tension in a muscle fiber. However, the conformational changes of F-actin during its interaction with myosin are still insufficiently studied. In the present study, the changes of the state of F-actin at HMM binding have been explored by the method of polarized UV-fluorescence microscopy.

Materials and methods. The study was carried out on glycerinated ghost single fibers of rabbit muscle[1,2]. Such fibers were free of myosin, troponin and tropomyosin[2,3] and contained more than 80% of actin[3]. In some experiments, the fibers were treated with 10% glutaraldehyde for 1 min. HMM was prepared by tryptic digestion of rabbit skeletal myosin using the method of Szent-Györgyi[4]. The Ca^{2+}-ATPase activity of HMM was 0.95 µmoles P_i/min/mg when measured at low salt concentration, pH 7.5 at 25 °C. F-actin was decorated with HMM by incubation of a ghost single fiber in a solution containing 5 mg/ml HMM, 60 mM KCl, 1 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.0. The intensity of fluorescence (I_m) and the degree of polarization of tryptophane fluorescence (P) was measured by polarized microfluorimetry[5]. P was registered at fiber orientations both parallel ($P_{\parallel}$) and perpendicular ($P_{\perp}$) to the plane of the exciting light. All measurements were