which (of a^p/a^p genotype) gave rise to non-pigmented tadpoles which contained pigmented clusters in their brains (fig. 3, d). No wild-type pigmentation occurred in those tadpoles other than that of egg origin.
Since presumably the same mechanism of egg pigment accumulation in the brain cavity occurs also in the wild-type tadpoles, it has been concluded that in all tadpoles in which the clusters occur, the clusters are composed of melanophages that accumulate the egg melanosomes.

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Electrophoretic heterogeneity exhibited by the S-allele specific glycoproteins of *Brassica*

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Summary. A number of self-incompatibility genotypes of *B. oleracea* were analyzed by sensitive electrophoretic procedures. Members of the class of S-allele specific glycoproteins that increase in correlation with the onset of the incompatibility response were resolved into several components on SDS gels. The implications of this molecular heterogeneity are discussed in relation to S locus function.

In Cruciferae, the pollen-stigma interaction of self-incompatibility is believed to be influenced, at least in stigma cells, by glycoproteins produced under the control of the S locus[2-4]. Elucidation of the structure of the S-specific glycoproteins is therefore crucial to the understanding of their role in cell-cell interactions at the stigma surface[5-7] and to a detailed molecular genetic analysis of the S locus. Our analysis of the aforementioned glycoproteins by iso-electric focusing (IEF) and by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) utilizing highly sensitive stains is reported here.
The homozygous self-incompatibility genotypes S_2, S_5, S_6, S_7, S_8, S_9, S_{11}, S_{13} and S_{14} of *Brassica oleracea* var. *acephala*, were obtained from the National Vegetable Research Station (NVRS) in Wellesbourne, U.K. (courtesy of Dr David Ockendon), and bear the station's allele designation. Genotypes S_2^c and S_{14}^c were isolated[8] at Cornell University by repeated inbreeding of homozygotes of *B. oleracea* var. *capitata*, and were shown by genetic analysis to correspond to the S_2 and S_{14} alleles of NVRS[9].
For analytical IEF, 25 flower buds were collected, often from a single plant, and their stigmas harvested. Pollen contamination was avoided by harvesting stigmas from buds at 1 day prior to anthesis; such stigmas are pollen-free and equivalent to flower stigmas in their incompatibility response. The harvested stigmas were frozen in liquid nitrogen, pulverized, dissolved in 50 µl of deionized water, and the homogenate centrifuged at 2 °C for 30 min at 10,000 × g. The resulting supernatant was assayed for total protein according to Bradford[10] with bovine gamma globulin as a standard (Bio-Rad Protein Assay, System I). Samples containing 200 µg of protein were loaded onto strips and focused for 2 h at 2 °C on pH 3.5–9.5 PAG plates (LKB). The plates were fixed and stained with fluorescein-labeled concanavalin A (Con A-FITC) according to Burridge[11]. Figure 1A shows a stained gel on which many Con A-binding bands are revealed, particularly in its acidic portion (pH 4–7). The bands that focus above pH 7.0 are well resolved at the concentration of stigmas used. Among these basic bands, some appear to be common to all genotypes albeit in varying concentrations. These common bands aside, the different S genotypes shown in figure 1A each exhibit a unique basic glycoprotein pattern, with one or more differential bands (white arrows). The S_{14}^c and S_{14} genotypes on the one hand, and the S_2^c and S_2 genotypes on the other, have very similar if not identical IEF patterns. This identity is explained by the fact that in each set, the 2 lines, which have different genetic backgrounds, nevertheless carry identical S alleles as explained above. This correlation of IEF pattern with S allele identity irrespective of genetic background, suggests the involvement of the S locus in the determination of these unique basic protein patterns. Moreover, the seemingly S-specific bands which can only be detected in the stigmatic and not in the stylar or anther tissues of the flower were functionally correlated to the self-incompatibility reaction in our analysis of stigmas at different stages of development. Immature buds at about 5 days prior to anthesis are self-compatible, and only

become self-incompatible at about 2 days prior to anthesis. The 2 stages can be readily distinguished by the increased size and the appearance of yellow petal pigments in the self-incompatible buds. Following electrophoresis of equivalent amounts of protein from the different stigma stages, ConA-FITC staining revealed trace levels of the differential IEF bands in the self-compatible immature phase, and a marked increase in their concentration at the onset of the self-incompatibility response, as shown in figure 1 for 3 representative genotypes. The concentration change is

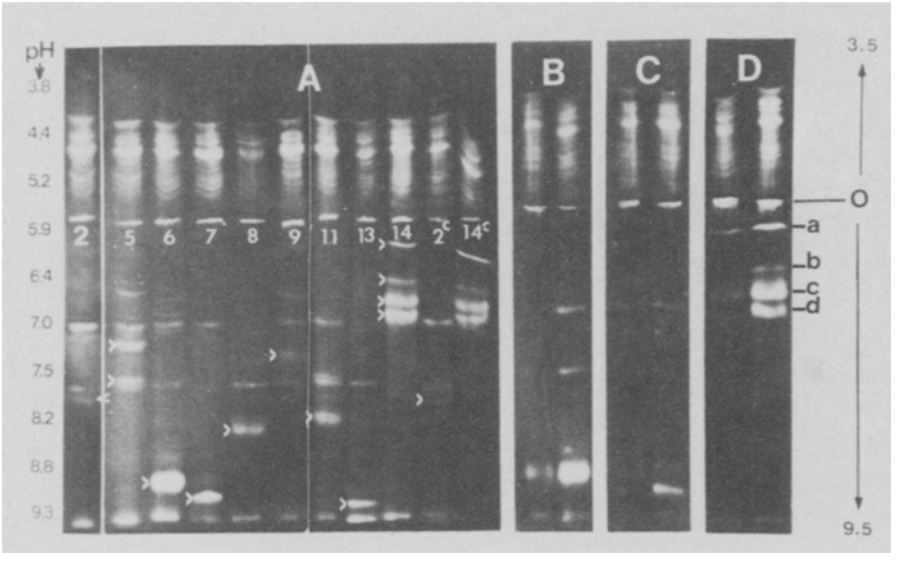

Figure 1. Isoelectric focusing patterns of stigma homogenates. Samples were loaded onto sample strips and positioned at 0. The pH gradient was determined with a surface pH electrode. The gels exhibited a pH range of 3.5–9.5 with the neutral zone being approximated by the position of band d. The gels were fixed, washed and brought to neutral pH in buffered saline, stained with ConA-FITC[11], and photographed over a UV light source. *A* Comparison of stigma extracts from different S genotypes. The numbers refer to the S allele for which they are homozygous. Arrowheads indicate the bands characteristic of the different S alleles. *B*, *C*, and *D* IEF patterns of stigma extracts from the self-compatible phase (left channel) and the self-incompatible phase (right channel). *B*, S_6; *C*, S_7; *D*, S_{14}.

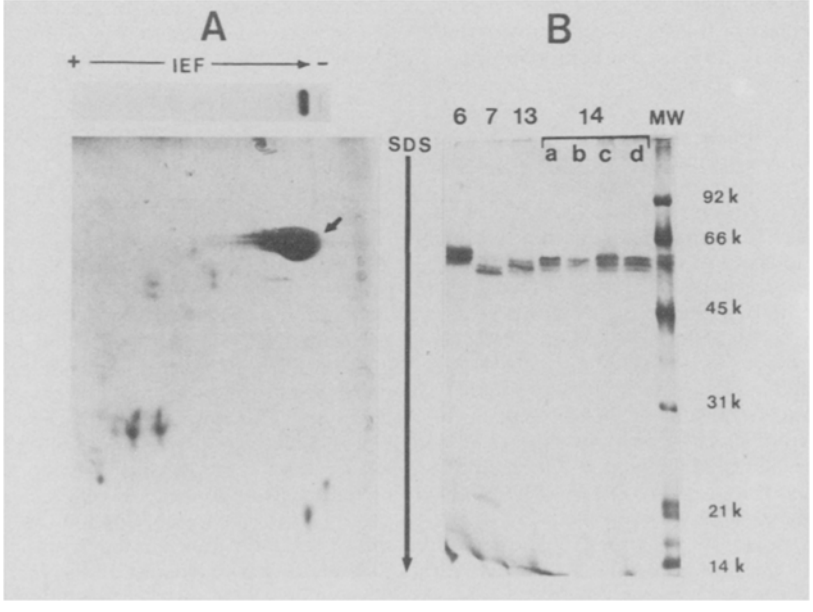

Figure 2. SDS-PAGE analysis of S-specific glycoproteins. Electrophoresis was according to Laemmli[13]. The stain was silver nitrate[14].
A Native 2-dimensional electrophoresis of S_6. The 1st dimension was run as in figure 1. The basic portion of the 1st dimension gel (illustrated by a Coomassie stained gel, top) was loaded onto a 10% (w/v) SDS gel, and secured in place with melted 1% (w/v) agarose in SDS sample buffer. Arrow points to the S_6 specific cluster of molecules. The low mol.wt species that focus in the same pH zone as the S_6-specific molecules do not bind ConA and are probably unrelated to the S system. *B* Eluates of S-specific IEF bands. The S-specific bands were isolated from IEF gels as follows: aliquots of a stigma homogenate were focused in parallel; one channel was stained with Coomassie Blue to visualize the separated bands; gel slices were cut from unstained channels at the level corresponding to the stained S-specific band; the proteins were eluted from the gel slices into 50 mM Tris buffer pH 7.2 by freezing and thawing, and the resulting solution was mixed with an equal volume of 2× SDS sample buffer. Numbers for each channel refer to the homozygous genotypes used: 6, S_6; 7, S_7; 13, S_{13}; 14, S_{14}. 14 (a, b, c, and d) refer to the 4 IEF bands shown in figure 1D. MW, molecular weight standards (Bio-Rad) with the indicated molecular weights.

clearly observed for the unique differential bands of S_6 (fig. 1B) and S_7 (fig. 1C), as well as for the multiple bands of S_{14} (labeled a, b, c, and d in fig. 1D).

In order to characterize the S specific bands further, they were analyzed by SDS-PAGE. In one set of experiments, native 2-dimensional electrophoresis was carried out by electrophoresing the basic portion of the 1st-dimension IEF gel into an SDS gel. The silver stained second-dimension pattern is shown in figure 2A for S_6 stigmas. The S_6-specific glycoprotein is resolved into several components, all clustered in the 60–66 k mol. wt region and well separated from the other dozen or so lower mol. wt protein species seen on the gel. All components indicated by the arrow (fig. 2A) can also be detected in self-compatible bud stigmas and exhibit the developmental change in concentration described above. In a 2nd set of experiments, the differential glycoprotein bands of the S_6, S_7, S_{13} and S_{14} homozygotes were analyzed by SDS-PAGE of eluates from unstained IEF gel slices corresponding to the bands in question. In all cases, the eluates of what appear on IEF gels as single glycoprotein bands were shown by silver staining to consist of several species on SDS gels (fig. 2B). For each genotype, the major components consist of several species all of which stain with ConA-FITC. The 4 unique bands (a, b, c, d in fig. 1) revealed by IEF in S_{14}(S^c_{14} are no exception. They too are each resolved into what appear to be identical complexes of several mol. wt species. Based on the intensity of staining with silver nitrate, 2 bands are prominent within each cluster; their estimated mol.wts are: for S_6, 65 k and 63 k; for S_7, 59 k and 57 k; for S_{13}, 63 k and 61 k; for S_{14}, 64 k and 62 k. Other workers[12] have estimated a mol.wt value of 57 k for an S_7 specific molecule, although no mol.wt heterogeneity was reported by them for this genotype.

The functionally related S-allele specific molecules that we have discussed here are also related in their molecular structure. Although differing greatly in pI points, from the very basic S_6, S_7 and S_{13} molecules (pI > 9.0) to the slightly acidic and neutral multiple IEF bands of S_{14} (pI 6.2–7.2), they are all resolved into several glycoprotein components of similar mol.wt on SDS gels. As this molecular heterogeneity may be the result of differences in amino acid and/or carbohydrate composition, it is not clear at this stage whether the SDS-resolved components are different subunits of a native multimeric S molecule or intermediate forms of the processing modification of a core polypeptide chain. Since the components in question are glycoproteins as demonstrated by their binding to ConA, post-translational modification by glycosylation must occur in any event. The question is thus raised as to whether the S locus codes for the core protein or controls the modifying enzymes. The answer to this question is clearly significant for the understanding of the molecular basis of the genetically determined S specificities and of cell-cell interactions in plants.

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Effects of thyroxine on the reproductive organs and growth of young chickens

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Summary. Male chicks of an egg-laying strain were injected with 0, 1, 2 or 4 µg/100 g b.wt thyroxine s.c. daily from 7 to 12 weeks of age. Increasing dose suppressed testicular development and puberty was completely blocked by the highest level. Live weight gain was enhanced by thyroxine treatment. Similar treatment of females prevented normal development of the ovaries at puberty.

It has been widely reported that administration of thyroid hormones, or their analogues, to mature males or females of several species, including the domestic chicken, causes regression of gonads and reduction of spermatogenesis and oogenesis[2]. There is also an inverse relationship between thyroid secretion and gonadal development during natural annual cycles in the domestic duck[3] and the Canada goose[4]. There is little information in the literature, however, of effects of thyroxine administered to young chicks on their sexual development, including histological studies of endocrine organs.

We have, therefore, studied the size, structure and development of gonads and other endocrine organs in chickens given several doses of thyroxine from 7 to 12 weeks after hatching.

Methods. Chicks of an egg-laying strain (Thornber 404, Mytholmroyd Hatcheries, Hebden Bridge) were penned in groups from the day after hatching and offered chick starter crumbs and water ad libitum in an environmental temperature of 35 °C and continuous light. From 3 weeks of age they were caged individually and offered a standard grower ration (Linton Mill Ltd, Wintringham, Malton) in a room at 22–25 °C with a 15.25 L:8.75 D lighting pattern, the light phase starting at 07.00 h.

Two experiments were carried out: a) 8 male birds aged 7 weeks were treated for 36 days, after which they were killed