THE VIGOUR AND MALE STERILITY OF HYBRIDS BETWEEN THE SPECIES *TRITURUS VULGARIS* AND *T. HELVETICUS*

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(Received May 1, 1959)

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Introduction

Some geographical variation will probably be discovered in all species. Though contemporary theories of the origin of species are inductions from the observation of such variation, the discussion involved has not yet coined a technical term for one taxonomic unit which is commonly encountered and theoretically important. A *Rassenkreis* or *polytypic species* is a group of conspecific subspecies (or geographical races) which replace one another spatially. An *Artenkreis* or *superspecies* is a group of congeneric species which replace one another spatially. The so far nameless unit is a congeneric group of forms spatially replacing one another, of which some are called species and some are called subspecies.

The importance of such a group is only appreciated when details concerning *both* taxonomic ranks are considered simultaneously, because first this permits an analysis of the differences between these categories, and secondly may suggest differences in their respective histories. Ring species are special cases of this mixed taxonomic situation.

The *Triturus* newts are well classified by traditional methods (Mertens & Muller 1940) and this classification can be used to present good, but simple, examples of the categories discussed (Mayr 1942). Study of these newts has therefore contributed to the contemporary hypothesis that "Speciation, in the sense of multiplication of species, proceeds in two steps. During the first step, a population (or subspecies or subspecies-group) becomes isolated by extrinsic barriers. Gene interchange with the parental population is completely or almost completely interrupted. During this period of separation the isolated population becomes genetically reconstructed. If this change of the genetic make up includes factors for reproductive isolation (isolating mechanisms), as well as for ecological compatibility, the isolated population may reach species level and can re-invade the range of the parental population" (Mayr 1951).

T. cristatus & *T. marmoratus* are two familiar and long recognised species (Angel 1946, Lantz 1947). Because the area in France in which both are found is relatively small compared with the ranges of both species, these can be grouped as an Artenkreis. *T. marmoratus* can however be compared with the subspecies of *T. cristatus*. It can be considered as a subspecies which has reached "species level" (according to the above theory) because the nature or the duration of its "period of isolation" permitted the inclusion of "factors for reproductive isolation" in the change of its "genetic make up".

During recent years spermatogenesis has been studied in four subspecies of *cristatus*, and in the nominate subspecies of *marmoratus* and in 7 of the 10 possible hybrid combinations between them (White 1946, Lantz and Callan 1954, Callan & Spurway 1951 and unpublished material, Spurway 1953).

These authors have found no multivalents in any male meiosis in any control material though these have been observed in a freshly caught specimen of the related *Salamandra* (Scholl *in* Hartmann 1953). Univalents have been very rare in control material, but all hybrid animals examined had reduced chromosome pairing and contained univalents. Multivalents have been observed in the F_1 or backcross individuals of six kinds of hybrids, the exception being that hybrid where multivalents might be least expected because the least amount of pairing was observed. Therefore translocations must have occurred during the process of genetic divergence. Also a larger number of translocations must have occurred during the process we call "speciation" than have occurred during the process we call "sub-speciation".

The present paper describes a comparable analysis of the meiosis of a single male hybrid between members of the species *Triturus vulgaris* and *T. helveticus*. These newts belong to a separate species-group which is morphologically so different from either *T. cristatus* or *T. marmoratus* that it had been separated from them into another genus (*Lissotriton*) during the early nineteenth century. Benazzi and Lepori (1949) made some hybrids between the Italian representatives of these species-groups by an artificial fertilization of *T. cristatus carnifex* ova by *T. vulgaris meridionalis* sperm. The disorganisation of spermatogenesis in this hybrid between species-groups was similar to those observed in than the intra-species-group hybrids, but it was more extreme, as would be expected.

This paper presents the evidence that *T. v. meridionalis* & *T. h. helveticus* also differ by

translocations. Therefore, this form of "genetic reconstruction" seems characteristic of speciation in the genus *Triturus*. Is just this precise change in genetic make-up likely to occur without itself constituting immediately an "isolating mechanism" i.e. are such cytological differences between taxonomic groups compatible with the contemporary hypothesis about the sequence of events in metazoan speciation ?

Taxonomy, Distribution and Phylogeny

The nominate subspecies of both *T. vulgaris* and *T. helveticus* are among the commonest vertebrates of Western Europe!

It is not difficult to distinguish between living females of the two species, but the differences in bone and muscle proportions by which this is performed are undescribed because the animals are too soft to be measured critically, and the differences in colouration vary between populations (Smith 1951). The most qualitative and verbalizable difference that the present authors recognise concerns the relations of the lips. The upper and lower lips of females of *T. v. vulgaris* meet precisely, whereas the upper lips of *T. h. helveticus* females overhang the lower and therefore are visible when the animals are laid on their backs. This greater broadness of the maxillary region compared with the mandibular region, like the larger eye and the greater ossification of the fronto-squamosal arch, contributes to the relatively greater width of the head of *T. h. helveticus*.

The males of the two species are differently coloured and while breeding develop conspicuous and different epigamic structures (Smith 1951). These differences are qualitative. Much of the colour disappears in preservative, and the structures do not redevelop to their full extent in captivity even when the males court females successfully. They will not be redescribed here as no segregation involving them has been observed.

That taxonomic description (as opposed to identification) can only be made of one sex, on freshly captured animals, for half the year at most, is less unfortunate than it might seem as the animals can only be captured systematically during the breeding season. During this period they congregate in still water and a pair is often captured together while courting (there is no amplexus or copulation). The species are usually found separately, but it is not rare to find a mixed population. In these, one species is usually rarer than the other, but which is the rarer species may differ from year to year in the same body of water. Reports of wild hybrids have not been accepted, and are not accepted by us.

The expectation that the visual epigamic differences contribute to the complete reproductive isolation between the two species is not supported by observations of their behaviour when together.

The elaborate courtships only differ quantitatively between the two species (Marquenie 1950) and therefore do not specifically display the different details of the patterns and structures. Though visually dramatic to a human observer this courtship seems to function by distributing skin secretions. Similarly it seems to be the males which distinguish between the females, again by using their sense of smell. A male frequently courts a non-conspecific female through a glass partition and ignores

her when she is transferred so that they are in the same tank. A male frequently dashes several inches to such a female but after touching her cloaca with his nose he walks away. One of us (H.S.) has seen a *T. h. helveticus* male court for 50 minutes a *T. v. vulgaris* female whose cloaca was blocked by a piece of faeces. Twice she was removed, and when first put back he immediately returned to her. But after the second removal when the faeces had been taken from her, he only nosed her and swam away.

The details of the animals' habitats show that they must have different ecological requirements. This is confirmed by their reactions to captivity. Individuals of *T. v. vulgaris* usually regress, not only their sexual behaviour, but their epigamic structures and their gonads and gonaducts within days, or even hours, of capture. This is so rapid that they are inconvenient both to photograph, and to use for artificial inseminations. They usually leave the water soon afterwards. Only one captive female (*v. Vulgaris* 1, Fig. 3) has been seen by us to return to water to breed next season.

Though wild *T. h. helveticus* newts usually disappear from the water after spawning they usually remain in it in captivity. They are likely to breed year after year in aquaria even though they do not redevelop their epigamic structures (Plate 2, Fig. 1).

Fig. 1 shows the geographical distribution of the different subspecies of these two species. It contains some corrections to that published by Spurway (1953). These are due to new evidence summarised by Freytag (1954). This new information suggests an explanation for the geographical distribution observed. Because the species are sympatric for such a large proportion of the range of *T. helveticus* they do not constitute a super-species according to the usual definition of this term.

In north Portugal the *helveticus*-newts are small. The male epigamic characters are typical but poorly developed and muted in this form which is called *h. sequirai*. (Plate 1, Fig. 1). The Italian *T. v. meridionalis* (Plate 1, Fig. 2) is similarly small and muted when compared with *T. v. vulgaris*. Freytag (1954) describes positive resemblances with *helveticus*-newts. In the Western Balkans and the Caucasus live a series of forms which possess both the epigamic coloration of *T. v. vulgaris* and the epigamic structures of *T. h. helveticus* well developed or even hypertrophied. *T. v. graecus* has the largest range of those forms which have been given names, but it too seems a small stunted version of a more northern form *T. v. tomasinii*. The skulls of these forms, which are considered part of the *vulgaris* Rassenkreis, are also intermediate. Recently Fühn & Freytag (1952) have discovered that the newts of Asiatic Turkey also show positive characters of both species and are not typical *v. vulgaris*, as was previously stated. The same authors have also found similar animals in the Transsylvanian mountains ecologically distinct from the typical form of the plains, whose range extends to the western shore of the Bosphorus.

The distribution shown on the map (Fig. 1) must be post-glacial. During the height of a glaciation it is reasonable to imagine the range of *T. h. helveticus* being much farther south in Spain and North Africa. The present northern limit of *T. v. vulgaris* is about 70° N latitude in Scandinavia (Freytag 1954)*. Therefore it is reasonable to suppose

* This is the northern limit recorded for Urodeles. Darlington (1957) records only the Arctic Circle for *Hynobius keyserlingii* in Siberia.

that during the last glaciation *T. v. vulgaris* lived very close to the southern limit of the ice, and that populations of this species followed the retreating sheet so as to colonise the North European plain, and to enter Ireland before the sea separated this island from Great Britain. Similarly during the height of a glaciation, the range of the present

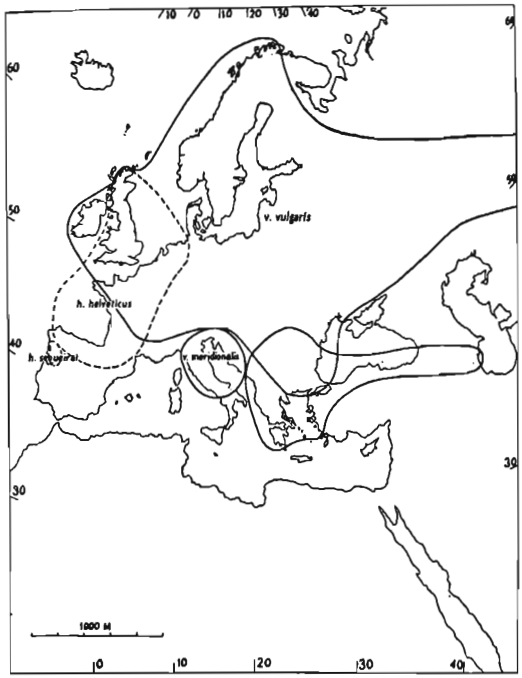

Fig. 1. Distribution of the subspecies of the polytypic species *Triturus helveticus*- - - and *Triturus vulgaris* ——. Only a single Balkan-Caucasian distribution is shown. This includes those of several separate but similar forms.

south-eastern forms would also be expected to have extended southwards along the eastern shores of the Mediterranean into North Africa. Here their ranges, or the ranges of forms like them, may have been contiguous with the range of newts which we would today recognise as *T. helveticus*. Such intermediate types may have formed part

of a mendelian population, i.e. been interbreeding, i.e. been conspecific, with both *T. vulgaris* and *T. helveticus*. Indeed such a doubly conspecific form or forms may be among those still living.

The discovery that *T. v. vulgaris* lives very near the tundra and also that an intermediate form inhabits Anatolia make it probable that the present distribution is a vestige of that of a ring species surrounding the Mediterranean, in which Haldane (1948)

Fig. 2. Suggested distribution of the hypothetical ring species during the glaciations. All the suggested land limits are tentative, but especially those to the north and east. For example here newts may have been confined to the Mediterranean littoral, at least during some periods, and there was perhaps a gap between the *vulgaris*-like forms and the *helveticus*-like forms extending some distance both north and south of the Pyrenees. The maximum extension of the Scandinavian ice sheet is shown.

would describe the *difference* between the two forms which are today sympatric in north west Europe as *specific*, and the differences between all the other forms as *subspecific* (Fig. 2).

The extension of *T. v. vulgaris* into most of the enormous area which it now inhabits, and the range of *T. h. helveticus* where it is today sympatric with *vulgaris* must have been post-glacial; the extinction or "retreat" of the hypothetical north African forms may also have been post-glacial, or it may have occurred during an interglacial. Indeed, the actual differentiation of the forms was probably consequent on climatic changes during the Pleistocene. There are two alternative theories; the differentiation was due (1) to the isolation of relatively large populations left as relicts during a contraction of range or (2) to the isolation of very small colonising populations (perhaps one inseminated female) during an expansion of range. As within the *cristatus-marmoratus* species groups the Pyrenees alone provided a "speciating" barrier.

Lantz (1934) and Freytag (1950) discuss hybrids previously made between these forms. Although *T. h. helveticus* and *T. v. vulgaris* have been hybridized in captivity, *helveticus* individuals mate more readily with *T. v. meridionalis* and the Balkan forms than with *T. v. vulgaris*. This is interpreted as evidence that these mediterranean populations are genetically less different from *T. helveticus* than *T. v. vulgaris*, confirming the greater morphological resemblance. However the sympatric forms live in the spatial context in which Dobzhanskian selection pressures would be expected to increase sexual isolation. An imitation of the experiment of Dobzhansky and Koller (1938) to discover if this isolation is less intense between animals caught in regions where only one species exists, cannot unfortunately be attempted, because of the physiological disturbance caused by transportation to individuals of *T. v. vulgaris*. It is probable that this greater facility of hybridization is due entirely to the fact that all the other subspecies of *T. vulgaris* breed more readily in captivity than the nominate subspecies. *T. h. helveticus* resembles them in this respect also. The comparable northern form of *T. cristatus* is similarly sterile in captivity compared with the populations of southern Europe. Hediger (1950) considers this to be characteristic of northern forms from temperate climates.

We are grateful to the late L. A. Lantz for the specimens of *T. h. sequirai* received from Oliveira do Duoro, Portugal during January 1946; and to Professor G. Montalenti of Naples for all the specimens of *T. v. meridionalis* which were brought to us from Rome by Professor G. Pontecorvo in April 1947. We received specimens of the nominate subspecies from, or were enabled to catch them by, John Godfrey, L. Haig, the late L. A. Lantz, the late J. W. Lester, L. C. Mandeville, N. M. Mitchison, and above all by Professor Marc de Larembourg and his colleagues at the University of Poitiers who organised many expeditions for one of us. The localities of the fertile individuals are entered on Table 1 except for the two *T. v. vulgaris* females which spawned in the same aquarium in 1943. These came from the western edge of the built-up area of greater London, one from the Surrey and the other from the Middlesex bank of the Thames.

Culture Methods and Life Histories

The viabilities of the various animals reared in our collection are shown in Table 1. This table shows that the culture conditions have never been satisfactory. Therefore no standardised routine has been developed. The first column shows the reference number of the family. This contains the year of breeding. The "19" is usually omitted in writing this date. The second column gives the duration of spawning,

which is *not* the duration of time during which the female or females spawned that season, *but* the duration of time during which were laid those actual eggs whose subsequent fate was recorded. Not all the spawn laid in family 54A during this period was kept. Eggs were not counted. The third column gives the locality data of the mothers, and the fourth that of the fathers, when the mating took place in captivity.

The larvae were fed on Daphnia and Tubifex. The age at which they were counted has been variable. Usually larvae were removed from the vessel where the spawn had been laid, or artificially fertilized, therefore a definite number was obtained. Sometimes at the beginning they were not counted, or only partially counted, so that an estimate only can be given in Table I. In the later families the spawn was removed weekly from the parental tank and kept at 20°C. The larvae from each week's batch were counted a fortnight after the first egg in that batch was seen to hatch. This routine revealed that there had been deaths during this period in 50B and 54A.

Metamorphosis took place in about 6 to 8 weeks. Each animal climbed out of the water with great speed, usually up the glass walls of the jar, though rafts were always provided, and aerial shoots of water plants were frequently present. Whatever their parentage these efts were about 3 cm long, which is small compared with wild animals. Usually metamorphosis was superficially complete, but quite commonly an animal with the adult jaws and eyelids but with a larval skin and crimson gills was found to have clambered out. If animals which had left the water were not immediately placed in a terrarium with standing water not more than 2 mm deep, they were found drowned when next inspected, sometimes within an hour, *whatever their developmental condition*. Such animals cannot adjust their specific gravity in water, and perhaps as a consequence they cannot swim. In Table I animals killed by such neglect are scored as "metamorphosing alive". Those first seen dead on the bottom are scored as "metamorphosing dead". Some of these must have been animals which were not noticed in time, though a greater frequency of them at first inspection in the morning is not revealed by the records written at the time. There is no evidence that an eft with gills has been removed from water prematurely, though many animals died during their first week on land, e.g. 51P. However in most families, though not in all, deaths continued to occur fairly steadily. The terrestrial efts were fed on wingless mutants of several Drosophila species and Enchytraea. Few grew to a length of 4 cm.

Death on land was often preceded by a sudden spurt in growth, the animals becoming very fat, not bloated, but with stiff bulky muscles, including those of the paired limbs. Their skins became wet and they returned to shallow water where they would feed. They did not develop tail fins and could not swim. Their cloacal lips became very swollen but did not differentiate sexually. They died within a month or six weeks of the beginning of this process. Such a pattern of development was particularly common to the specific hybrids made by artificial fertilization i.e. 50G, 50H and 50I. At one time it was thought to be peculiar to them.

The eight animals of the 1944 family of *T. v. vulgaris* whose sex became recognisable, differentiated while still terrestrial, four when twentythree months old and the remainder five months later. These animals were always small and had the short bodies

Table 1

Year & family	Duration of spawning	Locality data of mother(s)			Locality data of father if mating made in captivity			No. of larvae	No. metamorphosed alive	No. metamorphosed died	Last date seen	Notes on longest survivors
					v. vulgaris							
1943			two♀♀	April. 1943					15		12.11.46	terrestrial efts
1944	5 weeks	Chessington	1♀	7.4.43	Highgate	one♂	7.4.44	83	42	14	2. 1.51	two♂♂ five♀♀ non-functional aquatics
1950E	9 days	Aberlady	several♀♀	April 1950				74	47	1	1.5.52	puberty doubtful
1953E	4 days	Down House	one♀	24.5.53				20	19		liberated	
					v. meridionalis							
1947C	8 weeks	Rome	twelve♀♀	5.4.47				100+	38	22	23.2.48	terrestrial efts: epidemic
1949D	9 weeks	Rome	5♀	5.4.47	Rome	7♂	5.4.47	54+	20+	1	6.6.50	terrestrial efts: epidemic
					h. helveticus							
1942	larvae	Carradale		19.8.42				13	13		9.3.45	terrestrial efts
1947H	1 day	Camberley	three♀♀	June 1947				44	41	1	23.3.48	terrestrial efts
1950F	2 days	Ratho	several♀♀	April 1950				79	23	3	16.11.50	terrestrial efts
1954A	6 weeks	Bignoux	two♀♀	2.4.54				36	29	1	14.11.54	terrestrial efts
1954C	7 weeks	Nanjizel	21♀	3.4.53	Bignoux	22♂	2.4.54	49	42	2	14.11.54	terrestrial efts
					h. sequeirai							
1946E	6 weeks	Oliveira	two♀♀	Jan. 1946				7	2	1	16.6.47	terrestrial efts

Table 1 (*Continued*)

						v. meridionalis ♀ × *v. vulgaris* ♂							
1948A	4 weeks	Rome	2♀	5.4.47	Ruislip	31♂	14.3.48	11	11		13.4.49	terrestrial efts	
						h. sequirai ♀ × *h. helveticus* ♂							
1947G	4 weeks	Oliveira	1♀	Jan. 1946	Newdigate	7♂	22.5.47	33	26	3	3.2.50	aquatic efts	
						v. vulgaris ♀ × *h. helveticus* ♂							
1950H & I	art: fert:	Aberlady	several♀♀	April-May 1950	Ratho	several♂♂	April-May 1950	39	26	4	9.5.54	aquatic, puberty doubtful	
						h. helveticus ♀ × *v. vulgaris* ♂							
1950G	art: fert:	Ratho	several♀♀	April 1950	Aberlady	several♂♂	April 1950	78	68	1	9.5.54	aquatic, puberty doubtful	
						v. meridionalis ♀ × *h. helveticus* ♂							
1950B	10 weeks	Rome	3♀	5.4.47	Newdigate	7♂	22.5.47	3	3		2.7.57	one♂ two♀♀ functional	
1951P	2 weeks	Rome	3♀	5.4.47	Newdigate	14♂	30.4.51	1	1		2.8.51	had metamorphosed	
						(*v. meridionalis* ♀ × *h. helveticus* ♂) ♀ × *h. helveticus* ♂							
1954D	4 weeks		50B3♀		Nanjizel	18♂	3.4.53	4	3		1.6.56	terrestrial eft	

Further locality data concerning the members of the nominate subspecies

Chessington	Surrey	England	Bignoux	Vienne	France
Aberlady	East Lothian	Scotland	Nanjizel	Cornwall	England
Down House	Surrey	England	Highgate	London	England
Carradale	Argyll	Scotland	Ruislip	Middlesex	England
Camberley	Surrey	England	Newdigate	Surrey	England
Ratho	Midlothian	Scotland.			

and broad heads which are frequent in many vertebrates reared in captivity, presumably because of restricted space and malnutrition (Hediger 1950). These animals never developed their fins completely. They made no courting or ovipositing movements at all. Their parents were captured 16½ miles apart. Much of nineteenth century London, and the tidal river, were between these parental localities. *T. v. vulgaris* newts are common in the centre of the city, where they are habitually caught by children and often liberated a few weeks later. However during the 1939-1945 war, even in the centres of the big cities of western Europe, newts of this species appeared in stagnant water tanks and bomb craters the first spring after these had been created. Therefore there may be effective exchange between what in the breeding season would look like isolated populations.

The members of the specific hybrid family 50B from *T. v. meridionalis* 3♀ spontaneously inseminated by *T. h. helveticus* 7♂ were the only newts that functionally matured. The *T. v. meridionalis* mother laid very many eggs, and hatching was recorded in six of the ten groups into which these were divided by the collection of the spawn at weekly intervals. These newly-seen larvae were twice recorded as developmentally more advanced than usual, and non-hatching eggs also disintegrated more slowly than usual, suggesting that development had at least begun. Only three larvae survived to the first census at 14 days, two in the spawn of the first week and one in the seventh. These three animals climbed out of the water on the 43rd, 61st and 47th day after the first hatch in their respective age-groups. All of them had returned to water by the 28th October 1950 (i.e. in the year in which they were spawned) and fed under water a fortnight later. They were developing fins by the beginning of January 1951. On the morning of the 5th January 1951 all three animals suddenly appeared very similar to *T. h. helveticus*. Their head proportions and dorsal and flank pigmentation were *helveticus*-like. One of them made "low-intensity" but unmistakable courtship movements. No sign of sexual dimorphism could be seen. However by 18.30 on *the same evening* the three animals had developed differentiated genitalia. All animals had *helveticus*-like tail-tip and pigmentation, back ridges and the undifferentiated feet typical of *helveticus* when kept in captivity. The male 50B1♂ was 6 cm long with large black hedonic glands; 50B2♀ (7 cm) was sexually more differentiated than 50B3♀ (6·5 cm). The belly spots formed lateral bands on 50B2♀ and were scattered on 50B3♀. Both patterns are common in female *T. v. vulgaris* and *T. v. meridionalis* and are rare in *T. h. helveticus*.

We have never observed puberty in any other newt younger than one year old, nor have we elsewhere observed it to take the form of an explosive metamorphosis. This precocity seems an example of the hybrid vigour for which Dobzhansky (1950) wishes to use the Darwinian term *luxuriance* i.e. the result in the phenotype of heterosis* due to

* Most usages suggest that
the adjective *heterozygous* describes the *genotype*,
the adjective *heterotic* describes the *epigenotype* of Waddington (1957)
and the adjective *hybrid* describes the *phenotype*.
We will try to be consistent in our use of this interpretation of vocabulary (Spurway 1957 a & b).

heterozygosity which has *not* been evolved under the influence of the selective advantage conferred by that result (Wallace 1955).

Fig. 3 graphs the weeks during which over-wintered females spawned. The wild animals retained a spring spawning period as described by all previous authors. The January spawning of *T. h. helveticus* 19♀ in 1954 was while she was being courted by the hybrid 50B1♂, and the February spawnings of *T. v. vulgaris* 1♀ in 1944 and of *T. v. meridionalis* 1♀ in 1948 both followed an increase in the aquarium temperature by 12° C or more.

The late summer and winter spawning peculiar to the hybrid females 50B2 and 50B3 may be described as a disorganisation of canalization in time. That these hybrids have less *developmental* homeostasis than controls can be attributed to the fact that they belong to a population which is freshly synthesised and therefore cannot have begun to evolve *genetic* homeostasis (Lerner 1954).

The male 50B1 courted both his sisters and *T. h. helveticus* 19♀. No fertile spawn was produced nor would he have been expected to be fertile judging from his spermatogenesis (see below). Lantz (1934) however reports the hatching of an F_2 from such hybrids.

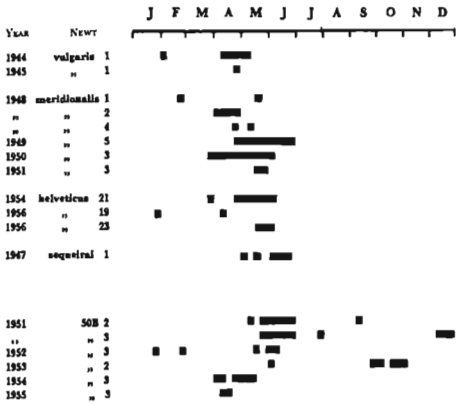

Fig. 3. Graph showing periods during which over-wintered females spawned. Horizontal axis represents time.

Only 50B3♀ ever became terrestrial spontaneously, though 50B1♂ became so after his operation in 1951. During this terrestrial phase black dorsal spots like those of the *T. v. meridionalis* mother became more conspicuous, as Lantz (1934) also reported. 50B1♂ died on 22.9.1955.

After producing fertile spawn in 1954, 50B3♀ became fat during a winter spent on land, the diameter of her body growing to about 2·5 cm. Part of this was filled with

functional ovary because she spawned during the spring of 1955 and her ovaries (as well as her fat bodies) were well developed on her death on 20.10.1955.

There was no sign of the stunting of growth such as was observed in the animals previously described. Plate 2, Fig. 2 is a photograph of 50B1♂ and 50B2♀ when both animals were sexually functional on 2.11.1953.

Cytological Method

A fragment of testis was removed and fixed while 50B1♂ was under ether anaesthesia on 16.9.1951, but this fragment proved to be full of spermatids and sperm and lacking in earlier stages of spermatogenesis. Again in 1952 on about the same date as in the previous year a testis fragment was removed; but this, too, proved to contain spermatids and sperm only. The operation was repeated for a third time on 22.7.1953, this time with success since, though much of the testis fragment contained spermatids and sperm, there were sufficient spermatocytes undergoing the metaphase of the first meiotic division for satisfactory cytological analysis.

The testes of newts belonging to the *cristatus* species group have been found to be in the best condition for the analysis of meiosis during the month of September. British specimens of *T. v. vulgaris* and *T. h. helveticus*, on the other hand, generally show a peak of meiosis in July; the F_1 hybrid male considered here evidently keeps to the same period. September has also proved to be too late for obtaining meiotic material from *T. vittatus*, another member of the *vulgaris* species group. It would therefore appear that one of the characters which differentiates the *vulgaris* from the *cristatus* species group is the earlier onset of male meiosis in the former.

The cytological technique employed was that which has proved satisfactory in previous work with newts. A fragment of testis was fixed for a few hours in 3:1 alcohol-acetic acid. A small portion of the fixed testis was subsequently tapped out on a microscope slide in aceto-carmine containing a trace of iron acetate. If cursory examination under the low power of a microscope showed dividing spermatocytes to be present, the material was immediately spread out over the slide, covered with a coverslip, covered again with a folded filter paper and pressed out firmly with finger pressure. The "squash" preparation was then inverted over a ridged dish containing 10% acetic acid. Provided that the coverslip is carefully cleaned after lying in acid alcohol before use, most of the squashed cells adhere to it rather than to the slide when separation takes place. The material on the coverslip was subsequently stained for ten minutes in iron aceto-carmine, then passed rapidly through acetic alcohol, alcohol and xylol prior to mounting in D.P.X. With newt material there is a technical advantage in staining in two stages: the cells flatten more readily when squashed after a very short period in aceto-carmine, a period which is too short for adequate staining. Hence the staining must be intensified subsequent to the separation of coverslip from slide.

Spermatogenesis in T. h. helveticus and T. v. meridionalis

The diploid chromosome number in both parent species of newt is 24. The chiasma frequencies of the two species are considerably lower than are those of the various

subspecies of *T. cristatus* but similar to the chiasma frequencies found in *T. marmoratus*. Twenty cells each from two specimens of *T. vulgaris meridionalis* and from two specimens of *T. helveticus helveticus* originating from Crail, Fife, Scotland were analysed at first meiotic metaphase. Frequency distributions of total chiasmata per cell are shown in Table 2. It might be objected that specimens of *T. h. helveticus* from Crail do not form

Table 2

Frequency distributions of total chiasmata per cell in T. vulgaris meridionalis *and* T. helveticus helveticus

Newt	Chiasmata per cell								Mean Chiasmata per cell	Standard Error of Mean ±	Variance
	21	22	23	24	25	26	27	28			
T. vulgaris meridionalis ♂ *a*	4	11	3	2					22·2	0·2	0·8
T. vulgaris meridionalis ♂ *b*	1		6	6	2	2	2	1	24·4	0·4	3·0
T. helveticus helveticus ♂ *a*		2	2	10	3	1	2		24·3	0·3	1·8
T. helveticus helveticus ♂ *b*	4	10	5	1					22·2	0·2	0·7

an adequate basis for comparison with an F_1 hybrid whose male parent was a specimen of *T. h. helveticus* originating from Newdigate, Surrey in South East England. However, the meiosis of males of *T. h. helveticus* from many different British localities has been found to be substantially uniform.

In both parent species the chiasmata at first meiotic metaphase are for the most part terminally localised. This can be seen in the camera lucida drawings of text-fig. 4 and in figures 1 and 2 of Plate 3. Although in most respects the meioses of these two species are very much alike, they differ from one another in two ways. First, as can be seen in the drawings and photographs, the meiotic chromosomes of *T. v. meridionalis* are systematically a little smaller than those of *T. h. helveticus*. Secondly, more chiasmata lie in strictly terminal positions at metaphase in *T. h. helveticus* than in *T. v. meridionalis*. Chiasmata were scored as non-terminal if distinct bulges were visible at the points of junction between bivalents and, of course, if more than one chiasma occurred within a single chromosome arm. *T. v. meridionalis* ♂*a* formed 171 non-terminal chiasmata in 20 cells, 5 of these being accounted for by chromosome arms united by two chiasmata. It may be compared with *T. h. helveticus*, ♂*b*, having the same chiasma frequency, in which the corresponding figures were 16 and 0. Similarly, *T. v. meridionalis* ♂*b* formed 249 non-terminal chiasmata, 34 being accounted for by chromosome arms united by two chiasmata. The corresponding figures for *T. h. helveticus* ♂*a*, having the same chiasma frequency, were 101 and 27. British specimens of *T. v. vulgaris* similarly form considerably more non-terminal chiasmata than do specimens of *T. h. helveticus*. No evidence is available to show whether this difference reflects a corresponding difference in the position of chiasmata at diplotene or whether it is due to

differing degrees of terminalisation between diplotene and metaphase. It is questionable, however, whether chiasma terminalisation occurs at all in newts.

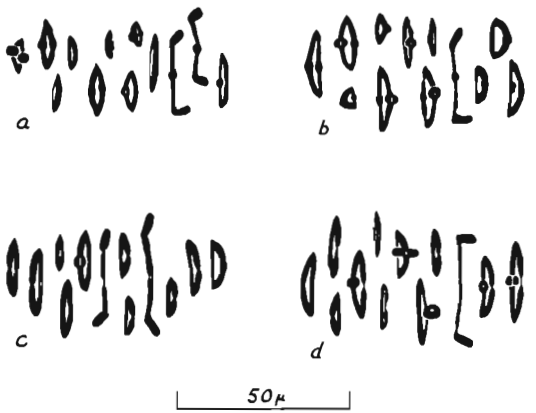

Fig. 4. First meiotic metaphase chromosomes in the parent newt species: (*a*) *Triturus vulgaris meridionalis*; 24 chiasmata (individual *a*). (*b*) *T. v. meridionalis*; 24 chiasmata (individual *b*). (*c*) *T. helveticus helveticus*; 22 chiasmata (individual *b*). (*d*) *T. h. helveticus*; 24 chiasmata (individual *a*). From squash preparations fixed in 3:1 alcohol-acetic acid and stained in iron aceto-carmine.

Spermatogenesis in 1950Bl♂

The analysis of chiasmata in this hybrid is based on a study of 110 cells at first meiotic metaphase. The frequency distribution is given in Table 3. As in all other newt hybrids which have been examined from this standpoint, the present hybrid shows a marked reduction in chiasma frequency, (mean 7·6 per cell), as compared with the parent species, which have mean chiasma frequencies of *circa* 22-24 per cell. The

Table 3

Frequency distribution of total chiasmata per cell in the F_1 *hybrid* 1950Bl♂

Newt	3	4	5	6	7	8	9	10	11	12	13	Mean Chiasmata per cell	Standard Error of Mean ±	Variance
1950Bl♂	1	4	11	14	23	24	15	11	3	3	1	7·6	0·3	3·8

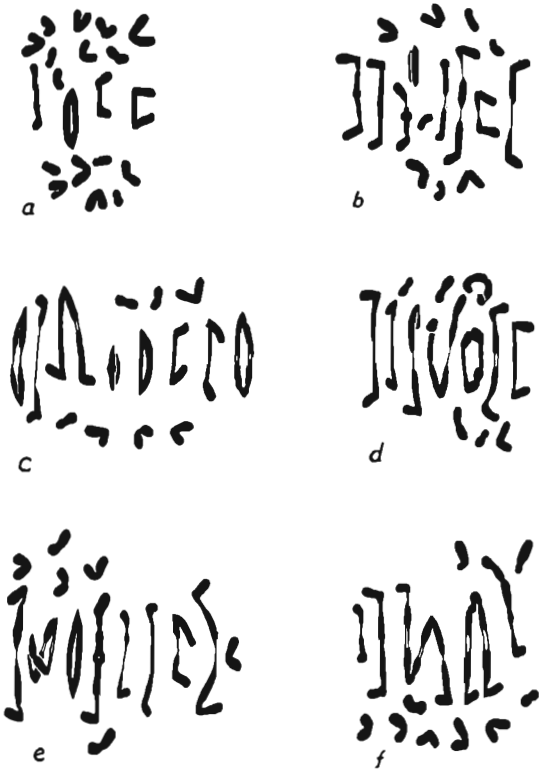

Fig. 5. First meiotic metaphase chromosomes in the interspecific F_1 newt hybrid 1950B1♂ (*Triturus vulgaris meridionalis* ♀× *T. h. helveticus* ♂): (*a*) 5 chiasmata, 16 univalents. (*b*) 9 chiasmata, 8 univalents. (*c*) 13 chiasmata, 1 trivalent, 7 univalents. (*d*) 11 chiasmata, 1 ring quadrivalent with non-disjunctional orientation, 1 trivalent, one of the bivalents unorientated, 5 univalents. (*e*) 12 chiasmata, 1 ring quadrivalent with disjunctional orientation, 6 univalents. (*f*) 9 chiasmata, 2 open quadrivalents, one with disjunctional, the other with non-disjunctional orientation, 1 trivalent incompletely orientated, 9 univalents. From squash preparations fixed in 3:1 alcohol-acetic acid and stained in iron aceto-carmine.

majority of the chiasmata are situated terminally. (See text-fig. 5 and Plate 3, Figures 3-6).

Well over half of the analysed spermatocytes show one or more multivalent chromosome configurations. The details are given in Table 4. The 110 cells contained 55 trivalents, but only 32 quadrivalents. There is an insignificant negative correlation of −0·04 between the number of trivalents and quadrivalents in a cell. Reading Table 4 diagonally we see that there were 15+27=42 cells indicating the presence of at least one translocation, 2+8+5=15 indicating the presence of at least two, and 2+1+2 or 5 indicating the presence of at least 3. There was no direct evidence of more than three translocations, nor of serial translocations involving more than four chromosomes,

Table 4

y	65	37	6	2	110
2	2	2	0	0	4
1	15	8	1	0	24
0	48	27	5	2	82
	0	1	2	3	*x*

x represents number of trivalents.
y represents number of quadrivalents.
Each box contains the number of appropriate nuclei. Totals at top and right.

unlike those found in the meiosis of one of the backcross interspecific hybrids from within the *cristatus* species group (Lantz & Callan 1954). Therefore 50B1♂ is heterozygous for at least three chromosome translocations. Moreover since 3 indubitable examples of closed ring quadrivalents (Text-fig. 5 d and e and Plate 3, Figure 5) have been observed, we may be sure that at least one of the translocations for which the hybrid is heterozygous is a *reciprocal interchange*, evidence for which was lacking from the earlier studies of hybrid newts.

If we assume that all the interchanges were reciprocal, we may ask whether the results suggest that there were only three such interchanges, or that the number was larger, six being the largest possible number. We wish to thank Professor J. B. S. Haldane for the following answer to this question.

First suppose that there were only 3 reciprocal interchanges, and let the probabilities that the pairs should yield a multivalent be p_1, p_2 and p_3 respectively. It is assumed that the formation of multivalents by the three pairs was independent. This is supported

by the very high value (35·4%) of the coefficient of variation of the chiasma frequency calculated from Table 4. As there were 87 multivalents our estimate of $p_1+p_2+p_3$ is $\frac{87}{110}$.

If $p_1+q_1=p_2+q_2=p_3+q_3=1$, the expected numbers of cells with:

no multivalents	is	$110\,q_1\,q_2\,q_3$
1	„	„ $110\,(p_1\,q_2\,q_3+p_2\,q_3\,q_1+p_3\,q_1\,q_2)$
2	„	„ $110\,(p_2\,p_3\,q_1+p_3\,p_1\,q_2+p_1\,p_2\,q_3)$
3	„	„ $110\,p_1\,p_2\,p_3$

If $p_1=p_2=p_3=p$ then $p=\frac{29}{110}$, and the expectations are $110\,q^3$, $330\,pq^2$, $330\,p^2q$ and $110p^3$. These are given in Table 5, 3rd row. If p_1 p_2 & p_3 were unequal, the expected number of cells with three multivalents is less than $110p^3$ or 2·01, and the expected number with no multivalents is also less. So the fit to the observed results is worse. For example let $p_1=\frac{17}{110}$ & $p_2=p_3=\frac{35}{110}$, whence the expected number of cells with 0, 1, 2, and 3 multivalents are 43·23, 48·25, 16·79, and 1·72 giving a χ^2 of 7·47.

Table 5

	0	1	2	3	>3	χ^2
Observed	48	42	15	5	0	
Expected, 3 interchanges	43·92	47·17	16·87	2·01	0	5·61
„ 4 „	45·57	44·92	16·61	2·73	0·17	2·09
„ 5 „	46·50	43·69	16·42	3·09	0·30	0·98
„ 6 „	47·10	42·42	16·29	3·30	0·89	0·32

Numbers of multivalents observed, and expected for different numbers of reciprocal interchanges.

Table 5 shows the number of multivalents to be expected if there had been 3, 4, 5 or 6 reciprocal interchanges, each of which generated multivalents with equal probability. As an example of the method of calculation:

If there were 5 interchanges

$$p=\frac{87}{550}\quad q=\frac{463}{550}$$

and $110\,q^5=46{\cdot}50$ while $5pq^4\times 110=43{\cdot}69$.

If the values of p_1, p_2 . . . . p_6 were unequal the expected frequency of cells with no multivalent would be less.

The values of χ^2 have been calculated after summing the expectations for three multivalents and over. Since the total 110, and the value of p (namely $\frac{87}{110}$ divided by the number of translocations) are fixed there are only 2 degrees of freedom.

The fit between the observed data and the expectations based on the hypothesis of 5 and 6 interchanges is very good.

If we consider the possibilities of 3 and over 3 multivalents separately, we find that $\chi^2 = 1{\cdot}99$ for 6 interchanges, which is still a very good fit. The possibility that there were only three interchanges is not excluded on the usual criteria of significance, which it must be remembered are not very accurate for small expectations. But the fit would be worse if, as is likely, the different interchanges produce multivalents with unequal frequencies. Therefore we can say that there were probably, but not certainly, more than three interchanges, and may well have been as many as six.

It is surprising to observe such a high frequency of multivalent chromosome associations when the mean chiasma frequency is as low as 7·6 per cell. The F_1 hybrid 1950J1♂ (*T. m. marmoratus* × *T. cristatus carnifex*) forms no multivalents at all at the higher mean chiasma frequency of 10·8 per cell (Lantz and Callan, 1954) yet it must be supposed to be heterozygous for translocations comparable in number to those in 1950B1♂. It may be that in the present hybrid the translocations involve greater chromosome lengths: alternatively, chiasmata in 1950B1♂ may be more closely restricted to terminal chromosome regions, a condition which would enhance the relative chances of formation of multivalents.

The orientation of the trivalent associations at first meiotic metaphase is generally of the convergent type, with two centromeres directed towards one pole, and the centromere of the middle chromosome, with chiasmata in both arms, directed towards the other pole. This was observed in 50 trivalents out of a total of 55. Of the remaining 5 trivalents, 2 showed linear orientation and in 3 the orientation was incompletely established.

22 of the 32 observed quadrivalents are in convergent (and hence disjunctional) orientation, whereas the remaining 10 quadrivalents are so orientated as to give non-disjunctional separation at anaphase. The regularity of trivalent convergent orientation is hence no guide to the frequency of quadrivalent non-disjunction.

No first meiotic metaphase of this hybrid showed less than 5 univalent chromosomes, mean univalent frequency per cell being 11·3, variance 7·3, standard error ±0·2. The univalents habitually come to lie at the spindle poles at full first metaphase. For the most part they are included in the telophase nuclei, though very occasionally lagging univalents give rise to micronuclei separate from the major nuclei of telophase. Diploid restitution nuclei have not been observed, nor is there any evidence of equational division of univalents at first anaphase.

The vast majority of the secondary spermatocytes must evidently contain unbalanced chromosome complements. Of 20 first meiotic anaphases only 5 showed twelve chromosomes at each pole. Yet in this individual mere possession of the normal haploid chromosome number is no guarantee of genetically balanced cells. Most of the spermatid nuclei containing twelve chromosomes must be genetically unbalanced by virtue of the non-disjunction of whole chromosomes and/or of parts of chromosomes which occurred during the first meiotic division. Yet 1950B1♂ produced a plentiful supply of spermatozoa. Cell degeneration is entirely post-meiotic in this hybrid and

roughly 50% of the spermatid nuclei fail to transform into mature spermatozoa. These degenerating cells persist, for a time at least, among the sperm bundles.

Ever since Muller and Settles (1927) demonstrated the inertness of lethals in Drosophila spermatozoa it has been usual to consider gamete degeneration as being dependent on genotypic incompetence of the parent organism and not of the gamete itself, and this view will be assumed in the discussion to explain the extreme susceptibility of gametogenesis to failure in a hybrid. However some genes intrinsic to the cells themselves would be expected to play some part in the actual process of the development of a spermatid into a spermatozoon, and examples of these have been recognised on the Y-chromosome of *Drosophila* species. Spermatid degeneration may in some way reveal genotypic unbalance in the small component of the haploid complement which has this function.

DISCUSSION

This paper presents data about an example of a familiar class of organism—the specific hybrids.

Family 50B was a typical hybrid family. In the menagerie environment provided, *all* members of family 50B were either much *more* or much *less* efficient both reproductively and somatically than members of the two species to which their parents belonged. Hybrid organisms are variously described as showing vigour, inviability, hypersexuality and sterility; and, as in family 50B, these contradictory abstractions not only segregate among members of the same sibship but are frequently manifested in one and the same individual.

Haldane (1955) has discussed some heteroses (epigenetic effects of heterozygosity, see Spurway 1957 a) and has emphasised that those which seem nearest the relevant gene actions are novelties either replacing, or additional to, the corresponding effects of the two homozygotes. When organs or tissues are considered, hypertrophy compared with the two control groups is a frequent heterosis, but such heteroses would not be observable in soluble substances and therefore we cannot say if they also occur among the primary products of gene actions. Nor can such exaggeration be the universal developmental effect of heterozygosity because any change in development must involve at least one compensatory process, which may be inconspicuous because it is merely an excretion of some precursor which has not been used up in the new developmental economy. Hybrid inviability is the technical term for disharmony between different heteroses occurring in different processes in the development of one individual. Such disharmony can occur among the epigenetic effects of a single genotype because heteroses like other gene actions are tissue specific, or, perhaps more accurately, function specific. Such hybrid inviability is more common in species and higher hybrids than in subspecific and population hybrids, probably because in the former more loci are heterozygous and *all* these heterozygosities are novel in the *immediate* phylogenetic history of the individual. The greater the number of unusual heteroses the less the likelihood that these can be integrated. Epigenetically there seems no difference

between euheterosis and luxuriance in Dobzhansky's (1950) sense. These words (which in form seem to describe the epigenotype if not the phenotype) are used by Dobzhansky to describe the *histories* in the population of the *genotypes* responsible. As Wallace (1955) perorates euheterosis can be considered as that fraction of the luxuriance which is compatible with the transmission of integrated genotypes through successive generations.

The integration of development itself is among the processes often heterotically hypertrophied—a hybrid often has increased phenotypic flexibility (Thoday 1953) developmental homeostasis (Lerner 1954), versatility (Haldane 1955) or homeorhesis (Waddington 1957). These phrases are provisional, but they are attempts to describe an objective attribute in non-emotive and non-value judgement language as a preliminary to quantifying it.

Family 50B is a typical species-hybrid family because the rare survivors of an enormous early mortality showed this quality to an exaggerated extent. The breeding of animals with such hybrid vigour has been considered economically worthwhile in many cultures, despite the difficulties of mating, pregnancy and parturition, and also the infantile death rate among the hybrids.

That hybrid vigour is most conspicuous in species hybrids suggests that it is positively correlated with the number of loci involved. This in turn suggests, though it does not prove, that alleles at all loci have heterotic properties though only those at a few loci may be integrated into euheterotic systems. That the luxuriance of the survivors is greatest among hybrids which also show the greatest infantile mortality suggests that the capacity for regulation itself may be enhanced in even the most extreme cases of hybrid inviability, but only rarely is this enhanced regulatory capacity sufficient to compensate for disruption due to differing heteroses of more specific and simpler processes.

Among commercially valuable hybrids the development of the reproductive system is frequently defective. This function is unnecessary for survival as is well known from the practices of castration and celibacy. Laboratory study has confirmed the popular* opinion that sterility is peculiarly characteristic of hybrids, and such study has also failed to detect any comparable defect common to many groups and similarly compatible with adult survival. Among laboratory mutants many affecting, for example, the integument and the special senses are known. No similar groups of phenotypes have been discovered among hybrids while in the shelter of captivity, which would not have been previously familiar solely because they would have been worthless for economic exploitation.

One generalisation has however been made about the sterility of species hybrids, and that too is exemplified by family 50B. In species hybrids one sex, almost always the heterogametic, is frequently more defective than the other. We do not know whether the male which was defective in family 50B is heterogametic in these species,

* One of us (H. S.) first heard of hybrid sterility because her father showed it to her as evidence that God so values order that he had invented a special auxiliary process to negate even occasional disorders. In a different terminology, hybrid sterility is an example of natural selection in its conservative aspect, which is most strikingly shown in centripetal selection within a species.

though Spurway (1945) had previously argued from very scrappy evidence that it is in *T. vulgaris*. 50Bl♂ is in more detail typical of such sterile hybrids. The development of his urinogenital system had apparently been normal, except for precocity, until the onset of meiosis. This and all subsequent processes were abnormal and variable from cell to cell within the tissue.

Meiosis is probably the commonest single climacteric for the onset of hybrid unbalance, and if the organism has survived and the gonad developed so far, this process is examined as a routine. Abnormality of meiosis in at least one sex is the minimum condition for making (or recognising) specific distinction between two parent forms.

Two causes of disturbed meiosis have been recognised, that leading to chromosomal sterility and that leading to genic sterility, and again 50Bl♂ was typical of metazoan hybrids in that both were operating. Chromosomal sterility results from lack of pairing homology between the chromosome sets of the two parents. As in 50Bl♂ this is frequently due to rearrangement of the chromosome material. Such rearrangement probably always precedes loss of homology by repeated mutation, which must also occur on theoretical grounds i.e., two allels must finally become so changed from one another that they lose their pairing affinity, though the distinction may be naive. As in 50Bl♂, the abnormal configurations observed often throw light on the history of taxonomic divergence. We shall return to this. Chromosomal sterility is thus a direct consequence of the actual way in which integrated genetic systems are transmitted. Given that chromosomes and the method of their reproduction have evolved, chromosomal sterility is likely if a hybrid is made and survives.

Genic sterility is comparable to any other hybrid failure (Stebbins 1958, Spurway 1957a, and b). It is due to the hybrid genotype being incapable of integrating heteroses within these transmitting processes. Is the commonness of genic sterility i.e. that this process fails more frequently than other systems of hybrid animals, also a function of the form of the transmitting process? Stebbins (1958) has pointed out that the commonest periods of development during which a hybrid system becomes inadequate all involve rapid nuclear division without interphase pauses. Most of these processes occur during very early development, but two involve the development of the gonad. The first is the specialization of cells within the gonad, and the second is meiosis where two consecutive nuclear divisions are telescoped.

Spurway (1957 a & b) has put forward another theory complementary, and in no sense conflicting with that of Stebbins, which explains the frequency with which genic sterility affects the actual process of meiosis as being a direct consequence of the form of the *non-chromosomal* structures of the haploid generation.

Unless it were a familiar phenomenon, our contemporary evolution theory would not have predicted genic sterility *a priori*. Phenotypically meiosis has been remarkably stable during evolution, and evolved its present form before the separation of animals from plants. This form is clearly recognised, and the deviations from it are both rare and uncontroversially secondarily evolved from it. Why should such a phenotypically invariable process be so genotypically variable? Why should genetic divergence behind this constant phenotype be so consistently associated with speciation,

that it has come to be regarded as the most important single criterion of the specific difference, at least in the metazoa ?

Is this sterility an isolating mechanism *secondarily* evolved in order to conserve integrated systems ? Dobzhansky in 1937 suggested (see 1951) that the occurrence of maladapted individuals would itself exert a selection pressure giving reproductive advantage to individuals which avoided the matings that produced them. Hybrid sterility is the most inefficient of such mechanisms since it levies the greatest cost (in the sense of Haldane 1958) on both parent populations. The hybrids are not only formed; thus removing gametes from both gene pools (or preferably gametic or genotypic pools), but these hybrids also grow up, thus competing maximally with those individuals which are potentially able to contribute (or able to contribute enormously more) to the two gametic pools.

However though hybrid sterility is an economically wasteful barrier between two species once they are well differentiated it can be argued that the sequence of events called speciation would have produced Dobzhanskian selection pressure leading to the evolution of hybrid sterility during an earlier period when the two populations were less genotypically different.

The first consequence of genotypic divergence between two populations is the segregation of epigenetically inadequate genotypes in the F_2 and backcross generations from hybrids between these two populations, i.e., hybrid breakdown. However this same genotypic divergence may at the same time produce hybrid vigour in the F_1. Therefore the F_1 will exercise no selection pressure against its own formation whereas the second generation will exercise such a pressure. Genic hybrid sterility is precisely a mechanism which hinders the production of a second generation while permitting the birth of a first. When the genotypic divergence has further increased so that it results in hybrid inviability, this hybrid inviability itself exercises the selection pressure which destroys the capacity for sexual recognition between members of the two populations i.e., it leads to ethological isolation. Though genic sterility has been found in hybrids between populations which show no ethological isolation (e.g., in hybrids between subspecies of *cristatus*-newts—Callan and Spurway 1951 and Spurway 1953) and may be presumed to evolve earlier in a speciation process, this argument is seen to be specious if we consider a Dobzhanskian selection pressure in operation.

Because individual a′ which mated with individual a″ leaves more progeny than individual a‴ which mated with individual b′ the gametic pool will contain proportionately more allels from both a′ and a″ than from a‴, and will include those relevant to the progeny obtaining sexual partners from population *a* more frequently than from population *b*. Only such allels that do *not* act by reducing the fertility of the progeny of a′ and a″ will survive further in population *a;* for example allels that act by altering the timing of the reproductive season, by altering the cruising range of the individuals, or by altering their reactions to other stimuli. Therefore a Dobzhanskian selection pressure can only lead to a sterility *conditional* on the nature of the sexual partners; it cannot lead to an unconditional sterility i.e., it cannot lead to the production of individuals incapable of reproduction with any partner.

The attributes resulting from Dobzhanskian selection pressures would be expected early in the reproductive process, as any such attribute that was a defect in a zygote would in each generation generate a selection pressure against the birth of individuals having it; the parents of any individuals which manifested such an attribute would by that event have themselves reduced Darwinian fitness. Because they similarly involve gametic wastage we are doubtful if any isolating mechanism acting after insemination but before fertilization is due to Dobzhanskian pressures, the effects of which we would expect to find entirely among the social reactions of the two populations, or of the relevant reactions of their pollinators (Spurway 1955). The chemical differences resulting in the various incompatibilities between maternal tissue and the male gametes which have entered it are probably pleiotropisms of differentiation which had arisen under other selection pressures.

If neither selection pressures to produce differences in the phenotype of gametogenesis, nor selection pressures to directly produce isolating mechanisms are responsible for the differences which cause genic sterility, why have these differences been evolved ? The clue may lie in the fact that very similar failures of gametogenesis are being discovered among the products of inbreeding, and to be the basis for the reduction in fertility which inbreeding produces, and which is also traditionally familiar (Rees 1955, Maynard Smith, Clarke, and Hollingsworth 1955). Therefore any destruction of *genetic* homeostasis, either by enlargement or contraction of the size of the population, seems to disturb the development of the gametes more than it disturbs the development of other tissues. This means that the gametogenic tissues have (by definition of terms) less *developmental* homeostasis than other tissues, and it is possible to see why this should be so.

The evolution of multicellular organisms is almost entirely a problem in what is today called developmental homeostasis. It is (1) the problem of evolving interaction among the products of developmental versatility in a population of cells each one of which has the same genotype i.e. battery of organisers; and it is also (2) the problem of producing on a very large* number of different biochemical bases a range of phenotypes standardized enough to have suggested the common or *specific* name. Among sexually reproducing multicellular organisms two with identical genotypes must be exceptional (e.g., monozygotic twins) and may never have occurred in the whole history of many species, especially of metazoa.

An isolated biochemical change is impossible in an organism i.e. all genes are pleiotropic, and it must be unusual for such pleiotropisms to be simply immediate single excretions. Harland (1936) and Muller (1942) discussing the multicellular plants and animals respectively, came to the conclusion that all genetic change has pleiotropisms in virtually every tissue, and that speciation has largely involved the reintegration of these pleiotropisms. The fact that structures which are indistinguishable in two species may develop abnormally in the hybrids between them shows that similar phenotypes are producible by different genotypes. Repeated backcrossing has often shown that this need not be a change in the primary gene product at a locus demonstrably

* e.g. with only 100 segregating loci and only one pair of allels at each there are 3^{100}, or 10^{48} genotypes (as compared with $10^{9.4}$ men).

associated with a phene or character, but is often a reorganisation of pleiotropisms during development.

There is the most intense selection (by definition all-or-none selection) on the optimal functioning of gametogenesis during what Mayr calls "genetic reconstruction". Nevertheless the process of gametogenesis seems to have the smallest range of genotypes compatible with functioning, i.e., both too heterozygous genotypes (such as are produced by hybridization) and too homozygous genotypes (produced by inbreeding) are not competent to organise perfect gametogenesis, though these same genotypes are responsible for normal somatic functioning. However the selection pressure on gametogenesis is in one important respect different from that on other structures and functions. *In the all-important aspect of function the gametogenic tissue is not multicellular.*

The haploid generation is a single cell in metazoa and often in metaphyta. Otherwise it is a very simple tissue. There is no reason for a defective *gamete* to continue functioning at all, since there is no intergametic cooperation or a very minimum of such cooperation. In a *tissue* any cell that can perform *any* single one of its functions is better than a cell that can perform no single one of these functions. In contrast the sooner a defective gamete is removed from the population the better.

Therefore the gametogenic tissue has in some important respects not been under the selection pressures which have led to the evolution of multicellularity, i.e. the selection pressures to minimise disruption of canalization due to ubiquitous genotypic change. In this respect the selection pressures acting on gametogenic tissue may be compared with those acting on a population of unicellular organisms, in which also, as far as we know, heterozygosity has much less selective advantage than in multicellular organisms.

Paramecium aurelia and *bursaria* are diploid. An ex-conjugant from a mating between two clones may be highly heterozygous, but after a few generations its progeny become homozygotes of many different genotypes as a result of autogamy. Natural selection is mainly between different homozygous genotypes and it is unlikely that autogamy would occur if its results were disadvantageous. It is therefore improbable that a heterozygote is often fitter than either of the corresponding homozygotes. This is intelligible if metazoan heterozygotes owe their increased fitness to some attribute demanded by multicellularity. A *Paramecium* also lives in an environment incomparably more uniform than the range of environments in which the various cells of the same metazoan must live and collaborate. Thus the efficient specialization of homozygotes may be more valuable than the versatility of heterozygotes. Many deaths usually follow autogamy (Sonneborn 1955). Many homozygous genotypes may be presumed to be unfit in any particular environment, and some are probably unfit in all environments. It is of course possible that other protozoa take advantage of increased fitness accruing from heterozygosity. But Paramecia at least are so organized as not to do so.

This argument for a relative independence of the gametogenic tissue from selection pressures for developmental homeostasis is supported by two facts. First, breakdown of canalization (often a spectacular increase of variation between the behaviour of the cells in a tissue) frequently begins abruptly at meiosis. Meiosis is precisely the

climacteric at which developmental homeostasis ceases to contribute to the efficiency of the *function* of the gametogenic tissue—meiosis is the process by which the gametogenic tissue ceases to function as a tissue and separates into unicellular gametes, or very simple haploid persons, each for itself and the devil take the hindmost. As in 50Bl♂ the later processes of gametogenesis become progressively more disorganised. The later failures are not a consequence of the earlier, they are independent processes i.e. the previous organisation of the gonadic tissue is breaking down, and all positive evidence prevents us from arguing that this is a consequence of the cells concerned themselves becoming haploid and therefore losing their heterozygosity.

Secondly, genic sterility seems ubiquitous in metazoa where the haploid generation is unicellular and is the gamete itself. Indeed in many species the female gamete is an abstraction, because the haploid pronucleus is never alone in a cell. Genic sterility is much less important in metaphyta, in which the female haploid generation always becomes multinucleate by mitotic division and the male haploid generation is frequently so, even though these generations in the higher plants are very simple and may not develop cell walls*.

Therefore genic sterility seems to be a direct consequence of the association of the haploid generation with a unicellular, or trivially multinucleate, structure independent of developmental integration at the tissue level. This means that genic sterility is a direct consequence of the sexual process itself i.e. a direct consequence of the form which fertilization has evolved in metazoa and some higher plants. Thus genic sterility can be compared with some completeness, with chromosomal sterility, which is equally a direct consequence of the evolution of chromosomes with their methods of pairing, dividing, and organisation in integrated sets, associated with cell divisions.

The processes often result in chromosomes rearranging among themselves the material of which they are composed. These structural rearrangements are usually disastrous

* The inability to emancipate the haploid generation completely from the necessity of nuclear co-operation may explain two other important differences between plants and animals. Firstly it may explain why plant ontogeny has remained simple, and precision of developmental homeostasis seems much less important among metaphyta than among metazoa (Stebbins 1950 p. 183). This is because the genotypes must retain this special integrative function in two very different persons and two ploidies, therefore certain specializations of the more complicated person could not become possible during the evolution of plants. This simplicity has in its turn permitted the evolution of greater diversity of the genetic systems of plants compared with animals (Stebbins 1950 p. 186).

Secondly the necessity for the genome of the haploid plant generation to function epigenetically, and perform mitosis, must have prevented an extreme exploitation of heterozygosity, i.e. it must have prevented the evolution of genomes which, though they are themselves epigenetically insufficient, maximise the beneficial heteroses of the maximum number of diploid combinations in the species. This may explain why bisexuality, probably the most efficient method of preserving heterozygosity, is the rule among metazoan species, though there are many hermaphroditic taxons; but much rarer, certainly not the rule, among plants despite its frequent evolution (called *dioecy*) and also despite the evolution of various other specializations which hinder self fertilization. Because in plants haploid genomes have themselves to control some epigeneses a conflict has been introduced preventing out and out exploitation of heterozygosity by the diploid persons. Therefore there has been a consequent muting of the effects of selection pressures that would otherwise have resulted in mating systems that ensured heterozygosity. Such a muting has been absent (or enormously less) in the evolution of animals, where selection acts virtually solely on diploid persons.

but sufficiently often they can be recognised as having been associated with some climacteric in the evolutionary divergence of populations. Hybrid 50Blσ is heterozygous for at least three translocations i.e. such rearrangement of material must have taken place several times in the process of divergence between *T. vulgaris* and *T. helveticus*.

Rearrangements of a similar kind have been discovered in the *cristatus-marmoratus* group of the same genus. All the F_1 hybrids between subspecies of *T. cristatus* which have so far been examined are known to be heterozygous for one or more chromosome translocations (Callan and Spurway, 1951, and unpublished). Although there is no direct evidence of the existence of such heterozygosity in F_1 interspecific hybrids between *T. cristatus* and *T. marmoratus*, unequivocal indirect proof of this condition is provided by certain backcross hybrids. The chromosome complements of *T. cristatus karelinii* and of *T. marmoratus* differ from one another by at least two double translocations (Lantz and Callan, 1954). Therefore the speciation in newts seems in some way correlated with the establishment of chromosome translocations, heterozygosity for which entails gross loss of fertility in the hybrids which have been studied.

We have previously discussed the significance of translocations in newts, and two alternative views have been put forward.

One suggestion requires the fixation by chance of translocation homozygosis within a very small boundary population, a population consisting, perhaps, of the offspring of a single inseminated female. This female, or her mate, had developed from a zygote involving the gamete in which the translocation had first occurred or could only be very few generations descended from this (Wright 1941). This series of events is followed by selection within such a population, leading to greater fitness in the local environment i.e. leading to the descendants of the founders becoming a colonizing population. If newts arrive later in such an environment, from the centre of distribution of the species; they fail to swamp and erode the characteristics of the local race owing to the relative infertility of the hybrids, which will be translocation heterozygotes. This is the view elaborated by Callan and Spurway, 1951, and by Spurway, 1953.

The discovery of serial translocation heterozygosis in the interspecific hybrids between *T. cristatus karelinii* and *T. marmoratus* led Lantz and Callan (1954) to put forward an alternative view that chromosome translocations might conceivably persist within a larger interbreeding population, despite Wright's contention to the contrary. They pointed out that by taking into account the terminal initiation of chromosome pairing characteristic of newts and a supposed regularity of disjunctional orientation of multivalent associations at first meiotic metaphase, translocation heterozygotes would suffer little or no loss of fertility, except for unbalanced gametes resulting from meiotic divisions in which chiasmata had formed in chromosome regions proximal to translocated segments. The regular disjunction of multivalents with such proximal chiasmata would not be assured. This genetic situation would be expected to depress recombination between the proximal regions of translocated and untranslocated chromosomes, and might become of selective advantage were mutations of value in particular environmental conditions to accumulate in these regions. Such "locked" gene sequences

within a single chromosome would become permanently associated with particular gene sequences in other chromosomes of the complement in the event of such other chromosomes exchanging segments in serial fashion with one of the chromosomes already involved in a segmental interchange.

Such a serial translocation system, as has already been mentioned, differentiates *T. cristatus karelinii* from *T. marmoratus:* and it seemed to demand an explanation in terms of selection.

Consideration of the evidence provided by the present hybrid 1950B1♂ supports in two ways, the induction that population differentiation involved a very small number of newts. First here the two parent species differ from one another not by serial translocations but by at least three, perhaps six, separate single translocations, one at least demonstrably reciprocal. Thus recombination amongst the six pairs of translocated and untranslocated chromosomes is possible, at least in theory. Secondly, quadrivalents do not regularly assume disjunctional orientation at first meiotic metaphase. Provided always that what we observe in this hybrid holds good also for a newly arisen translocation within a species, we must accept the cogency of Wright's argument that translocation heterozygosis leads to infertility, and thus that translocations are unlikely to persist and become established within a large interbreeding population, such as would become separated by an alteration of range due to climatic changes.

Chance establishment of translocation homozygosis within a small peripheral population, followed by the selection of a local race barricaded by translocation against genetic erosion due to the advent of later arrivals from the centre of distribution of the species, appears to offer a more plausible explanation of our observations, and therefore in the two most successful subdivisions of the genus *Triturus* speciation seems to have been initiated during a period of foundation of small colonies, most probably during expansion of range.

SUMMARY

The geographical range of the two Urodele species *Triturus vulgaris* and *Triturus helveticus* is described. Both are polytypic. It is suggested that these species are the relict of a ring species previously surrounding the Mediterranean. This ring has been ruptured to the south in Africa and perhaps West Asia, but the animals have extended their range very widely in northern Europe and Siberia since the extreme glaciation of the Pleistocene.

The members of these two species reared in captivity are discussed. Only one family of three individuals reached sexual maturity. This family 50B were specific hybrids and they are presented as examples of extreme hybrid vigour. The one male courted but produced no offspring. Both females spawned but during an abnormally long period of the year. One accepted a male *T. h. helveticus*, and her backcross offspring survived metamorphosis.

The parent subspecies *T. vulgaris meridionalis* and *T. helveticus helveticus* form about 23 chiasmata per spermatocyte. The chiasmata are terminally localised to a somewhat greater degree in *T. h. helveticus* than in *T. v. meridionalis.*

The F_1 male hybrid between these two species has a mean chiasma frequency of $7{\cdot}6 \pm 0{\cdot}3$ per spermatocyte, chiasmata being terminally localised as in the parents. The mean univalent frequency per spermatocyte is $11{\cdot}3 \pm 0{\cdot}2$.

Over half of the first meiotic metaphases of the hybrid show multivalent chromosome associations. This animal is heterozygous for at least three and perhaps six single translocations, one at least a reciprocal interchange. About two-thirds of the quadrivalents are orientated disjunctionally.

Despite a very high proportion of genetically unbalanced spermatid nuclei, roughly half of the spermatids transform into superficially normal spermatozoa. Cell degeneration is entirely post-meiotic in this individual.

It is argued that the disturbances to gametogenesis, involving both chromosomal and genic sterility, are consequences of the pattern which the sexual process has evolved. Chromosomal sterility is a direct consequence of the structure of the chromosomes. Genic sterility affecting meiosis and after is a direct consequence of the gametes being unicellular; and therefore their later development has not been under as intense selection for developmental homeostasis as has multicellular tissue.

The significance of chromosome translocation for the history of newt speciation is considered. The present evidence supports the theory that translocations become established within small colonising populations which were the founders of the populations to-day recognised by taxonomy.

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PLATE I

Fig. 1. *Triturus helveticus sequirai* 4♂, 1♀

Fig. 2. *Triturus vulgaris meridionalis* 7♂, 5♀

Photographed by L. C. MANDEVILLE 11. 6. 1949

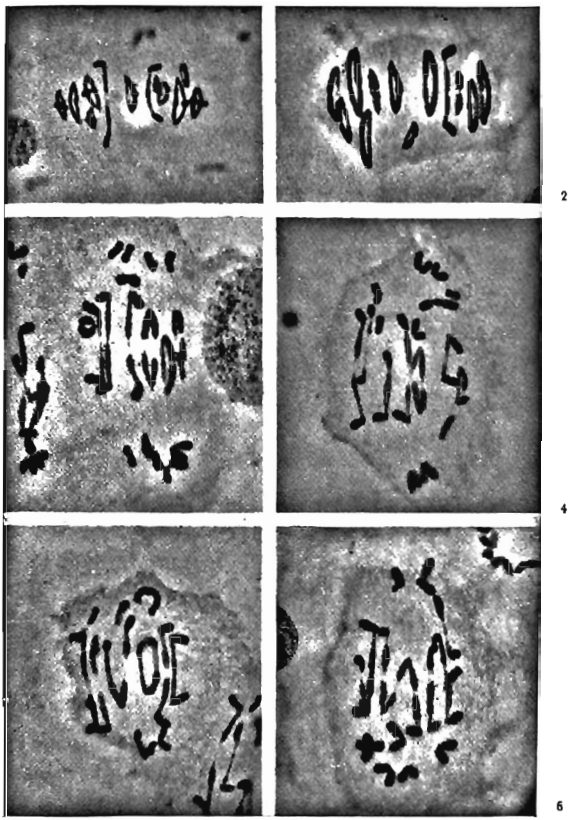
PLATE 2

Fig. 1. *Triturus helveticus helveticus* 18 ♂, 19 ♀ Nanjitel.

Fig. 2. hybrids 50B1 ♂, 2♀.

Photographed by W. Brackenbury 2.11.1953

PLATE 3



'Squash' preparations of newt first meiotic metaphase, fixed in 3:1 alcohol-acetic acid and stained in iron aceto-carmine. All × 450.

Fig. 1. *Triturus vulgaris meridionalis*, individual *a*, 23 chiasmata.
Fig. 2. *T. helveticus helveticus*, individual *b*, 23 chiasmata.
Fig. 3. F_1 hybrid 1950B1♂; 9 chiasmata, 12 univalents.
Fig. 4. F_1 hybrid 1950B1♂; 8 chiasmata, 1 open quadrivalent with disjunctional orientation, 10 univalents.
Fig. 5. F_1 hybrid 1950B1♂; 11 chiasmata, 1 ring quadrivalent with non-disjunctional orientation, 1 trivalent, one of the bivalents unorientated, 5 univalents.
Fig. 6. F_1 hybrid 1950B1♂; 9 chiasmata, 2 open quadrivalents one with disjunctional, the other with non-disjunctional orientation, 1 trivalent incompletely orientated, 9 univalents.