Chromosome Number of *Rorippa (Nasturtium) sylvestris*

THE only published count of the chromosome number of *Rorippa sylvestris* (L.) Besser (= *Nasturtium sylvestre* (L.)R.Br.) appears to be that of Manton[1], who found $2n = 32$. In a search for the other parent species which, with *Nasturtium officinale*, has given rise to the allotetraploid species *N. uniseriatum*[2], I studied a specimen of *R. sylvestris* obtained from the Newry canal at Newry (border of Co. Down and Co. Armagh, N. Ireland) and found this plant to have a chromosome number of $n = 24$ and $2n = 48$. Similarly, specimens of *R. sylvestris* from Horton-in-Ribblesdale (Yorkshire) and from the Botanic Gardens at Cambridge and Kew (the plant was growing as a weed in both gardens) were all found to have a chromosome number of $n = 24$. There thus seems no doubt that British specimens of *R. sylvestris* have a chromosome number of $2n = 48$ and not $2n = 32$ as reported by Manton.

Prof. Manton obtained her specimen of *R. sylvestris* as seeds labelled *Nasturtium lippizense* from the Munich Botanic Gardens. There is a single sheet of the plant of which the chromosome number was counted by Manton in the University of Manchester Herbarium. Unfortunately, it has no fruits, and the separate fruits which have also been preserved are not adequate for determining whether Manton's plant really was *R. sylvestris*. Also *N. lippizense* is listed as a distinct species in the Kew Index, and is not a synonym for *N. sylvestre*. It is thus possible that Manton's count does not refer to *R. sylvestris*, but to the European species *N. lippizense*.

Both the cuttings of the single plant from Newry and the clone of *R. sylvestris* growing in the Cambridge Botanic Gardens produced no seeds by natural pollination. A high set of good seeds was, however, obtained by bud pollination or by crossing the Newry and Cambridge plants. It thus seems that *R. sylvestris* is self-incompatible. This is rather unexpected in a hexaploid species, the basic chromosome number in the genus *Rorippa* being 8.

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[1] Manton, I., *Ann. Bot.*, **46**, 509 (1932).
[2] Howard, H. W., and Manton, I., *Ann. Bot.*, n.s. **10**, 1 (1946).

Amniotic Inoculation of Chick Embryos

THE respiratory tract of the developing chick embryo is susceptible to infection with various bacteria and the viruses of influenza, psittacosis, herpes and certain other infections of man and animals. This infection of the respiratory system is secured most readily by an inoculation of the virus directly into the amniotic cavity. Various techniques have been devised for this purpose, by Goodpasture, Hirst and others, but the most popular is probably that of Burnet[1]. By this method, virus is inoculated under direct vision into the amniotic cavity, which seems preferable to methods where the inoculation is made 'blind'.

In the course of studies on the reaction of the respiratory system to amniotic inoculation of influenza virus (to be published), it was discovered that Burnet's method could be simplified. The method that I have used seems to be such an obvious modification of Burnet's technique that doubtless other workers have come to use a similar method, but I have not seen any references to the use of such a procedure. I have decided to publish this note as there seems to be an impression that amniotic inoculation is difficult; but this need not be so, and the technique deserves wider application in experimental work.

Eggs of 13–14 days are candled, and the site of the densest area of the embryo marked with a pencilled cross. An equilateral triangle, with sides about 1 cm., is then drilled in the shell with a rotating disk, operated by a foot or electrically-driven dental drill. The shell in this area is then lightly dabbed with methylated spirit, and when this has dried, the triangle is gently levered off with a mounted dissecting needle sterilized by flaming. The shell membrane is now exposed, and should be undamaged. A drop of sterile saline is then placed on the shell membrane and a small opening made with a dissecting needle. The drop is coaxed to run under the shell membrane to separate it from the underlying vascular, easily damaged, chorio-allantois. A pair of delicate forceps, without teeth, is then used to tear away gently the triangular area of shell membrane. This can easily be done without damaging the chorio-allantois, especially as it is usually found that by this time it has dropped down some little distance. A heated dissecting needle is now used to make a small 'nick' in the chorio-allantois. The heated needle will make a short tear, and at the same time seal any opened vessels, thus minimizing bleeding.

A pair of sterile fine curved forceps is now passed through the tear in the chorio-allantois. The forceps are then opened and the underlying amnion grasped and pulled through the tear. The inoculation is then made into the amniotic cavity through a delicate short-bevelled needle attached to a 1 c.c. syringe held in the other hand. On completion, the amnion slips back into place. Sometimes, especially in older embryos, the embryo can easily be seen under the chorio-allantois as soon as the shell membrane is reflected. If so, it is not necessary to cut the chorio-allantois, as the inoculation can be made simply by passing the needle through the chorio-allantois and amnion into the amniotic cavity.

The triangle in the shell is then ringed round with a mixture of molten 'Vaseline' (with a little added hard paraffin) applied by a pasteur pipette. A sterile coverslip is quickly flamed and applied to the 'wall' of 'Vaseline'. Before placing in the incubator, the egg should be held level with the eye, to make certain that there is no gap in the 'Vaseline' ring. The embryo should be observed daily, and if still alive vigorous movements will be seen.

The only important modification in this method is that I do not find it necessary to 'drop' the chorio-allantois artificially by applying suction to a hole in the air-sac end of the egg.

Various authors who have used amniotic inoculation speak of a mortality-rate in the embryo, from the effects of the inoculation *per se*, of 30–40 per cent. I have not yet inoculated sufficient embryos to give a definite figure, but I have on a number of occasions inoculated a batch of up to six with sterile broth, and