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HYBRID INVIABILITY BETWEEN *RANA PIPIENS* FROM WISCONSIN AND MEXICO[1]

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Laboratory crosses among individuals of various populations of the widely-distributed meadow frog, *Rana pipiens,* have revealed the extent to which intraspecific gene exchange is possible. The occurrence of abnormalities in hybrids between certain geographical populations of *R. pipiens* has been demonstrated by Moore (1949a). In general, the intensity of hybrid defects is inversely proportional to the north-south distance between the localities of the parents employed in the laboratory hybridizations. It has also been shown (Moore, 1949b) that the geographically extreme northern and southern populations of this species differ in embryonic rate of development and embryonic temperature tolerance. Moore has inferred that these embryological differences represent differential adaptations to environmental temperatures, and he further surmises that the embryonic defects in hybrids between northern and southern individuals may be associated with the different temperature adaptations of the parental eggs.

The most severe abnormalities have been observed in hybrids between *R. pipiens* from Alburg, Vermont and two localities in eastern Mexico, Monterrey in Neuvo Leon and Axtla in San Luis Potosi (Moore, 1947).

In the present investigation, meadow frogs obtained simultaneously from a different northern locality, Oshkosh, Wisconsin and several localities in the State of Tamaulipas, Mexico provided the opportunity to hybridize the respective groups, and thus contribute additional information on the problem of geographical differentiation and the development of isolating mechanisms in populations of *R. pipiens*.

Experimental

A comparison was made of the rate of embryonic development and embryonic temperature tolerance of *R. pipiens* from Mexico and Wisconsin to determine if differences existed, and reciprocal hybrid crosses were conducted to ascertain the extent of developmental compatibility. The methods of experimentation were essentially similar to those employed in other work of this type (Moore, 1946, 1949b; Volpe, 1952, 1953). The technique, and the modifications introduced, will be brought out during the discussion of the experiments.

Embryonic temperature adaptations.—Ovulation of Mexican and Wisconsin meadow frogs was induced by pituitary injection. The

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eggs were stripped from the females into sperm suspensions prepared by macerating the testes of a male in 10-20 cc. of 0.1% Ringer's solution. Eight artificial fertilizations, in groups of two, were conducted. Each fertilization of a Mexican ♀ X Mexican ♂ was carried out simultaneously with a cross Wisconsin ♀ X Wisconsin ♂. By conducting parallel fertilizations, differences in rate of development of Mexican and Wisconsin embryos were readily apparent. The four ovulated Mexican females were derived from four different localities, as follows: (1) Storm's Ranch, 7 km. northeast of Gomez Farias, Tamaulipas; (2) La Union, 9½ km. north of Gomez Farias, Tamaulipas; (3) El-Mante-Tampico Highway, 29 km. east of El Mante, Tamaulipas; (4) El Mante-Tampico Highway, 72 km. east of El Mante, Tamaulipas. All four Wisconsin mating pairs employed came from Oshkosh, Wisconsin.

The egg masses from any single fertilization were cut into small clusters of 9 to 13 eggs to permit more surface for respiration. The eggs clusters were placed in finger bowls, each finger bowl containing ten clusters (referred to as a "group") in 200 cc of 0.1% Ringer's solution. The finger bowls were than distributed to constant temperature units, which maintained temperatures accurate to ±0.2°C. Development of four groups of Wisconsin eggs and four groups of Mexican eggs was observed at 18.9°C., 21.1°C., 27.5°C., 29.5°C., and 33°C.

The eggs derived from the Mexican females were larger than those obtained from the Wisconsin females. Measurements of the eggs (vitellus diameter) prior to first cleavage were made by means of an ocular micrometer calibrated from a 1/100 millimeter-ruled stage micrometer. The diameters of 86 Wisconsin eggs ranged from 1.60 mm. to 1.95 mm., the mean and standard deviation being 1.73±0.08. Similar measurements of 101 Mexican eggs indicated a range of 1.95 mm. to 2.20 mm. (2.10±0.06). The difference is significant (the difference of the means is three times the standard error of the difference). Although a few of the Wisconsin eggs approached the size of the Mexican eggs, a *cluster* of the latter could be readily distinguished with the unaided eye from a *cluster* of the former. This detectable difference in egg size (recall the relation of the volume of a sphere to its radius) made it possible to keep both the Mexican and Wisconsin clusters of eggs in the same finger bowl containing Ringer's solution at a desired temperature. This procedure reduced the error of developmental deviations due to any temperature variations which may have been encountered if each group of eggs had been kept in separate finger bowls. No difficulty was experienced in identifying the two groups of eggs while they developed within the jelly membranes. To obviate any difficulty in later development, the embryos of each group just prior to hatching were separated into individual finger bowls containing fresh 0.1% Ringer's solution which previously had been brought to the desired temperature.

TABLE 1.

A Comparison of Development in Eggs of *Rana pipiens* from Mexico and Wisconson

Age in hours between Stages 3 and 20

Experiment 1 18.9°C.			Experiment 2 18.9°C.			Experiment 3 21.1°C.			Experiment 4 21.1°C.		
Age in Hours	La Union, Mex. (110)[a]	Oshk., Wisc. (104)	Age in Hours	El Mante 29 km. e, Mex. (115)	Oshk., Wisc. (111)	Age in Hours	Storms Ranch, Mex. (111)	Oshk., Wisc. (105)	Age in Hours	El Mante 72 km. e, Mex. (116)	Oshk., Wisc. (109)
0	3[b]	3	0	3	3	0	3	3	0	3	3
11.0	7	7	18.2	9	9	6.0	7	7	8.2	8	8
13.2	8	8	24.5	10L	11E	13.5	9	9	18.2	11E	10M
16.2	9	9	31.2	12E	12L	16.5	10E	10E	24.2	12M	12E
20.5	10E[c]	10E	40.5	13E	13M	23.2	12E	11M	29.0	13E	12L
32.5	12M	12L	44.0	13L	14E	26.5	12L	12M	31.5	13M	13E
38.5	12L	13E	46.5	14E	14M	38.7	14L	14M	35.0	14M	14E
44.5	13L	14E	49.2	14M	14L	41.0	16E	14L	47.2	16L	16M
56.7	16E	16M	52.2	15	16E	45.5	16M	16E-M	55.0	17E-M	17E
64.0	16L	17E	64.5	16L	17E	50.5	16L	16M	66.2	18M-L	18E-M
70.5	17M	17L	70.5	17M	17L	64.0	18M	18E	69.5	18L	18M
81.0	18E	18M	76.7	17L	18E	71.5	19E	18L	78.0	19M-L	19E
93.0	19E	19M	91.2	18L	19E	76.2	19L	19E	80.0	20E	19L
96.7	19M	19L	95.0	19E	19M-L	79.0	20E	19L	83.0	—	20E
101.0	19L	20E	98.5	19M	19L	82.5	—	20E			
105.0	20E	—	102.0	19L	20E						
			104.7	20E	—						

[a] The number in parentheses indicates the number of embryos examined.

[b] The embryos were considered to be in a given stage when 50 percent or more of them exhibited the characteristics for that stage.

[c] The stages are described in Pollister and Moore (1937); the letters **E**, **M**, and **L** signify early, middle and late.

The rate of development was determined by examining the groups of embryos at each of the constant temperatures at frequent intervals from first cleavage to gill circulation. Certain problems arise in obtaining an adequate measure of the time required by a group of embryos to reach a given stage. Even when special precautions are taken to provide as uniform an environment as possible, eggs within a group exhibit variation in the speed with which they reach a given point in development. Thus, to permit statistical comparison between two groups of developing eggs, it would be desirable to make repeated observations to determine when 100 percent of the eggs had reached a particular morphological stage. This is laborious and tedious and even if this procedure were practical, it would be necessary to break the continuous embryonic development into a series of extremely minute discontinuous steps to permit an accurate determination of the extent of variation. The problem was attacked by selecting morphological stages at which recognizable specific processes begin, and the onset of a given stage was taken as the time at which 50 percent or more of the eggs within a group entered that stage. The stages drawn up by Pollister and Moore (1937) were employed, with the further refinement of designating the stages as early (E), middle (M), and late (L). In comparing two groups of eggs which were developing at different speeds, an estimate of the variability was obtained by noting the percent of embryos in the more slowly developing group which were as advanced as the majority of embryos in the second group which exhibited the faster rate of development. In all experiments, it was found that only five percent or less of the more slowly developing group of embryos were as advanced as the majority (50 percent or more) of the faster developing group of embryos; nor were there more than five percent of the faster developing group of embryos in as early a stage as the majority (50 percent or more) of the more slowly developing group of embryos. Moreover, the arbitrary criterion of 50 percent was found to be a conservative figure. From fertilization to stage 17 (tail bud stage) 80 percent or more of the embryos reached a given stage simultaneously; from stage 18 (muscular contraction) onward, 70 percent or more of the embryos entered a particular stage at the same time. Thus, although it is difficult to analyse the spectrum of variation, the ascertainment at any one reading of the norm of a group and the extreme variants of that group (in relation to the other group being compared) permits an accurate determination of comparative rates of development.

No differences could be detected in the rate of development of the four groups of Mexican embryos at each of the experimental temperatures. However, the groups of Mexican embryos differed in rate of development from those derived from Wisconsin. In Table 1 a comparison is made of the time in hours required by 50 percent or more Mexican and Wisconsin embryos within a group to reach the experimental end-point, stage 20, at two temperatures, 18.9°C

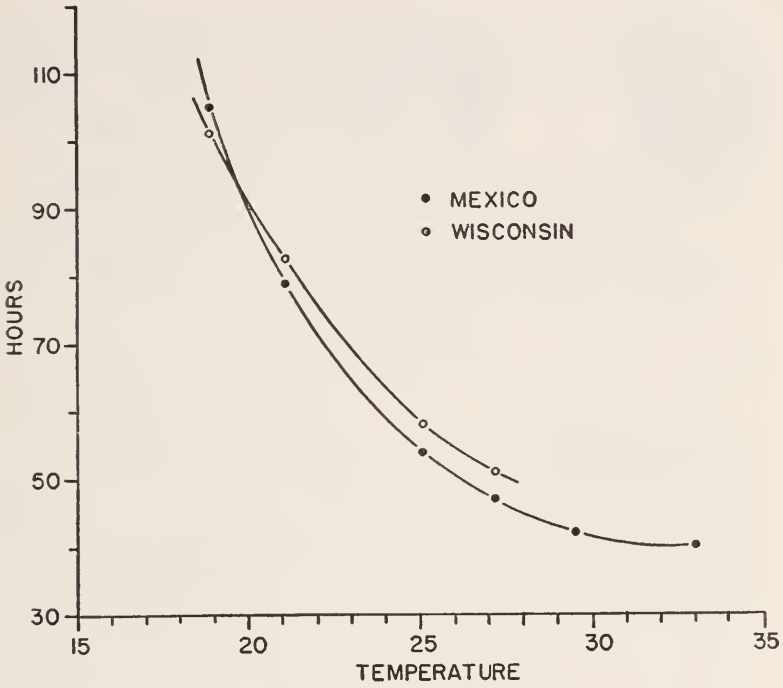

Figure 1. A comparison of the mean time, in hours, between stages 3 and 20 at different temperatures, in degrees Centigrade, of embryos of *Rana pipiens* from Mexico and Wisconsin.

and 21.1°C. At 18.9°C, the embryos from each of the different localities in Mexico developed more slowly than the Wisconsin embryos. At 21.1°C. (and higher temperatures), the Mexican embryos developed more rapidly. Figure 1 shows a plot of the mean time in hours between stages 3 and 20 required by four groups of Mexican embryos and four groups of Wisconsin embryos at the different temperatures employed. The faster rate of development of Mexican embryos at higher temperatures may be associated with the greater tolerance of these embryos to high temperatures.

To estimate temperature tolerance, records were made of the types and proportions of embryonic abnormalities at each temperature. The Mexican embryos are decidedly better adapted to the higher temperatures. At 33.0°C, early death of all of the Wisconsin embryos was evidenced by irregular cleavage furrows. At this same temperature, the Mexican eggs cleaved normally, but the majority of the eggs later exhibited defects in gastrulation. However, an average of 13 percent for the four groups of embryos (57 of a total of 437 eggs) developed to the gill circulation stage (stage 20). At 29.5°C,

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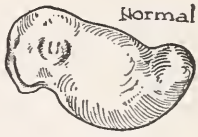

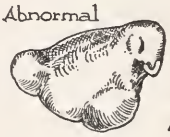

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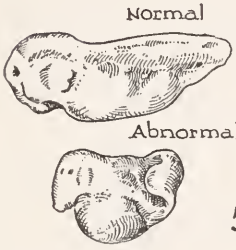

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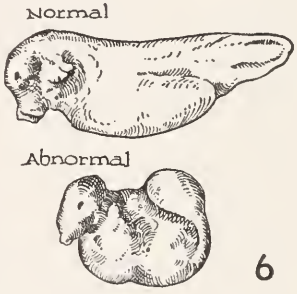

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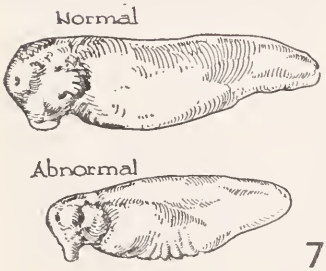

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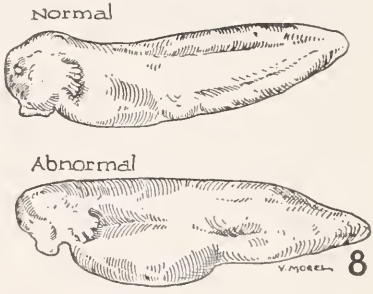

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Figures 2-8. Development of hybrid embryos from the cross La Union, Mexico ♀ X Oshkosh, Wisconsin ♂. Control embryos (La Union, Mexico ♀ X La Union, Mexico ♂) are shown in some figures for comparison. For full explanations, see text. **2.** The pitted appearance of the ectodermal surface of an early hybrid gastrula; **3.** The furrow encircling the hybrid egg and the narrow depression on the dorsal side representing the neural groove; **4.** *Top:* normal appearance of the tail-bud stage (stage 17) in a control embryo; *Bottom:* hybrid embryo with extreme reduction of head, fused oral suckers projecting from head, and the ventral abdominal bulge; **5.** *Top:* heart beat stage (stage 19) in a control embryo; *Bottom:* shrivelled appearance of hybrid embryo showing abbreviated head,

only a few developmental irregularities (total average of nine percent for four groups of embryos) were noted in the Mexican embryos. In contrast, the Wisconsin embryos developed only as far as the neurula stage. Moreover, development was normal in the Mexican embryos at 27.5°C, whereas 85 percent (390 of a total of 460 eggs) of the Wisconsin tadpoles were distorted.

At the low temperature extreme, adequate temperature control was not available. However, some indication of the differential effects of low temperature on the embryos was obtained by placing the developing eggs in a refrigerator which fluctuated from 6°C to 9°C. Four groups of Mexican eggs (total of 392 eggs) failed to cleave and 436 Wisconsin eggs developed normally up to stage 17 (300 hours), at which time the experiment was terminated. It appears that the embryos of *R. pipiens* from Wisconsin behave like those of other northerly distributed amphibians in being able to tolerate low temperatures.

In all probability, the differences in rate of development and temperature tolerance of Mexican and Wisconsin eggs represent adjustments that adapt the eggs to their respective environments. However, data on prevailing environmental conditions, particularly temperatures of their breeding waters, are lacking. Also, it seems reasonable to suspect that the improper interadjustments of morphogenetic movements observed in hybrid development (discussed below) are attributable, at least in part, to conflicting modes of action of temperature-related processes.

Reciprocal artificial hybridizations.—The procedure in artificial hybridization consisted of fertilizing the eggs of one female from a particular locality in two batches: the first with the sperm of a male derived from the same locality ("control"), and the second with the sperm of a male from a different locality ("hybrid"). Eight hybrid crosses were conducted, four of which involved a Mexican *pipiens* egg and a Wisconsin *pipiens* sperm each with controls ("Type A" cross); the others were reciprocal crosses each with controls ("Type B" cross). The hybrid embryos from the two types of crosses were

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ventral abdominal bulge, and the curved tail (the confining inner jelly membrane is not shown); **6.** *Top:* gill circulation stage (stage 20) in a control embryo which has hatched from the jelly membranes; *Bottom:* hybrid embryo showing little progress made over previous stage (fig. 5); the hybrid embryo is still trapped within the inner jelly membrane, but this is not shown in the illustration; **7.** *Top:* gill circulation stage (stage 20) in a control embryo; *Bottom:* an exceptional hybrid embryo which has hatched, showing the wrinkled blastocoele roof in the ventral abdominal region and the microcephalic condition with the downward projecting fused oral suckers; **8.** *Top:* tail fin circulation stage (stage 22) in a control embryo; *Bottom:* a surviving hybrid embryo in which considerable reorganization has taken place: the ventral abdominal bulge has disappeared and the head tends toward a normal condition; however, mouth and eye formations are atypical.

characterized by retarded developmental rates and extensive structural abnormalities. Within each type of cross the results were essentially similar; thus only a single cross of each type will be described in detail.

Type A, Cross 1:

La Union, Mexico ♀ X Oshkosh, Wisconsin ♂
La Union, Mexico ♀ X La Union, Mexico ♂ (Control)
Conducted at 18.9°C.

The rate of development in the early cleavage stages was maternal and the cleavage furrows were normal. The first indication of hybrid abnormality appeared during gastrulation. The surface of the presumptive ectoderm area overlying the blastocoele became wrinkled and presented a pitted appearance (fig. 2). During the closure of the blastopore and the subsequent formation of the neural folds, the series of pits arranged themselves in the form of a groove or furrow encircling the ventral portion of the egg (fig. 3). The groove marked externally the floor of the blastocoele. This was determined by puncturing a few eggs with a needle slightly below the groove. Blastocoele fluid escaped and yolk cells occupied the internal area at the level delimited externally by the groove. Thus the blastocoele failed to become completely obliterated during the gastrular movements. The persistent blastocoele later assumed the shape of a bulge in the ventral abdominal region of the developing hybrid embryos. The neural folds that formed in the hybrid embryos were narrower and closer together than in the control embryos. This condition foreshadowed the future reduction of the head. Figure 4 shows the extreme reduction and abnormalities of the anterior region of hybrid embryos at the tail bud stage. The oral suckers were greatly reduced in size and fused; the olfactory pits were absent or, if present, approached one another toward the mid-line; the stomadeal groove was absent; the gill plate showed no visible differentiation into arches; and the ventral abdominal bulge filled with blastocoele fluid was prominent. By late stage 19, the control embryos had already hatched (fig. 5). The majority of the hybrid embryos remained curled within the inner jelly membrane and failed to hatch. The structural abnormalities in the hybrid embryos were pronounced. The ventral abdominal bulge remained relatively large; the fused oral suckers projected downward; no stomadeal pit was present; and the gill plates remained undifferentiated and drawn close to the head. The hybrid embryos appeared arrested at this time. When the controls reached stage 20 (gill circulation), no advances were made by the hybrid embryos over the prior stage (fig. 6). Most of them cytolyzed within the inner jelly mass. However, some of the hybrids hatched. In those hatching, either the bulge in the ventral ectoderm became wrinkled and cytolysis ensued, or, in a very small percent, the bulge became reduced in size (fig. 7). Of the latter a few continued development into stage 22 (fig. 8). In these surviving hybrid embryos, the small fused suckers began to disappear, but the development of the mouth structures and eyes were much delayed and small.

These hybrids were transferred to a balanced aquarium, but all failed to live. Death was associated with reduced heads, abnormal eyes and atypical mouthparts, as control embryos placed in the same tank at the same time continued developing.

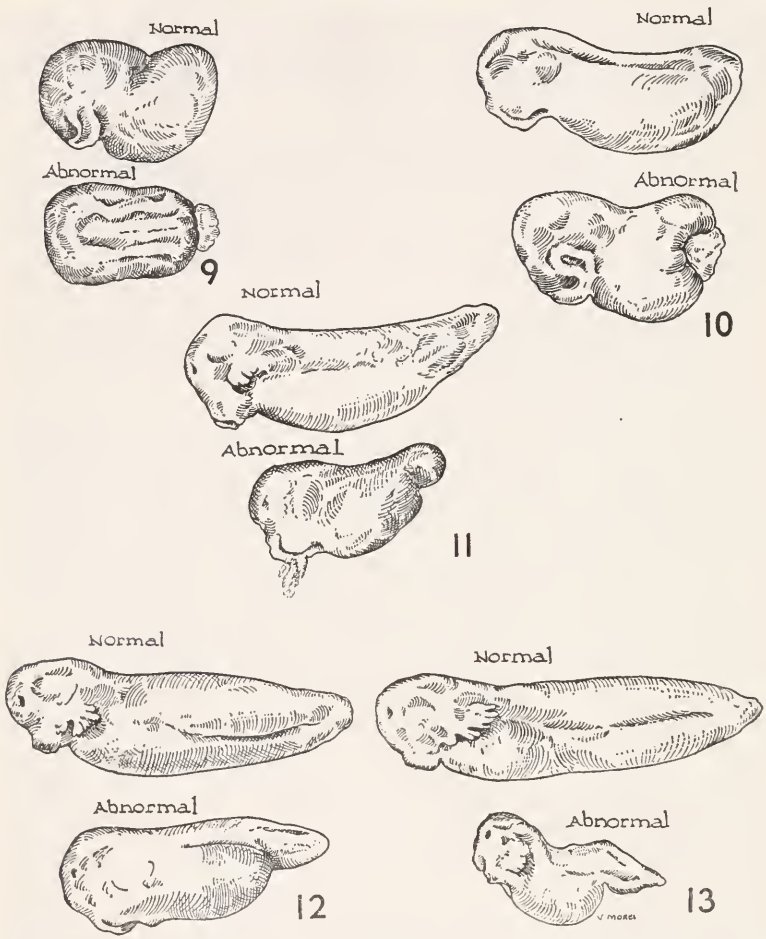
The hybrid abnormalities described above were also noticed in the other crosses of Mexican ♀ X Wisconsin ♂ ("Type A"). Hybrid embryos of this type of cross characteristically exhibited reduced heads with fused suckers and fused olfactory pits. In each of the four "Type A" crosses conducted, all hybrid embryos died.

Type B, Cross 1:

Oshkosh, Wisconsin ♀ X 29 km. e. El Mante, Mexico ♂
Oshkosh, Wisconsin ♀ X Oshkosh, Wisconsin ♂ (Control)
Conducted at 18.9°C.

The defects observed in these hybrid embryos were different, in fact almost opposite to those described in the "Type A" cross. Many embryos failed to recover from the effects of abnormal gastrulation. The disintegrating gastrulae exhibited open blastopores with extruding yolk plugs. However, a fair percent formed neural plates though the blastopore remained open (fig. 9). The neural folds that developed in the anterior end of the hybrid embryos were more divergent than encountered in the controls. This foreshadowed the enlarged head that appeared in later development. As shown in figure 9, the hybrid embryos were markedly retarded in development (the controls were in late stage 16). During later development (fig. 10), the hybrid embryos showed open blastopores of varied sizes. An atypical tail bud developed from the median dorsal lip of the open blastopore. Characteristic defects, other than the persistent yolk plug and shortened tail, were the enormous head, the abbreviated body form, and the unusually immense, widely-separated oral suckers. When the controls reached stage 19 (fig. 11), the surviving hybrid embryos were considerably retarded. An unnatural mucus mass filled the space between the huge oral suckers. As the controls progressed to stage 20 (fig. 12) the hybrids showed little developmental advance. The oral suckers remained large and divergent, and the blastopore beneath a shortened tail remained open in most embryos. By the time the controls were in stage 22 (fig. 13) all the hybrid embryos died. Cytolysis appeared to begin first in the yolk cells of the exposed yolk plug. The cytolyzing embryos showed enlarged heads, immense oral suckers, curved tails, and poorly differentiated gill plates. A few, prior to death, showed the earliest beginnings of external gills, but corpuscles were never observed coursing through them.

The hybrid embryos in the three other "Type B" crosses were characterized by the same kind and intensity of abnormalities. Not a single functional tadpole was obtained in all the crosses involving Wisconsin eggs and Mexican sperm.



Figures 9-13. Development of hybrid embryos from the cross Oshkosh, Wisconsin ♀ X Tampico, Mexico ♂. Control embryos (Oshkosh, Wisconsin ♀ X Oshkosh, Wisconsin ♂) are shown in all figures for comparison. For full explanation, see text. **9.** *Top:* a control embryo approaching tail bud stage (stage 17); *Bottom:* retarded hybrid embryo in medullary plate stage (stage 13), showing the open blastopore with the large, extruding yolk plug; **10.** *Top:* tail bud stage (stage 17) in a control embryo; *Bottom:* hybrid embryo showing the exposed yolk plug and the large, divergent oral suckers; **11.** *Top:* heart beat stage (stage 19) in a control embryo; *Bottom:* hybrid embryo with an enlarged head, stubby tail, and a thick mucus mass projecting from the overactive oral suckers; **12.** *Top:* gill circulation stage (stage 20) in a control embryo; *Bottom:* a hybrid embryo arrested in development; abnormal in those respects previously noted; **13.** *Top:* tail fin circulation stage (stage 22) in a control embryo; *Bottom:* appearance of a hybrid embryo just prior to cytolysis; note the enlarged head, shortened body form, and poorly differentiated tail.

Discussion

It is clear that isolation through hybrid inviability has reached a high intensity between Oshkosh, Wisconsin *Rana pipiens* and those from the area of Tamaulipas, Mexico. Moore (1947) demonstrated a similar high degree of incompatibility between Alburg, Vermont and eastern Mexico meadow frogs. It may have been argued that the observed hybrid abnormalities were due to certain characteristics unique only to Vermont meadow frogs among northern *pipiens* or to an equally peculiar property of those meadow frogs from the particular Mexican localities studied by Moore. Similar results in this investigation utilizing *pipiens* from another northern locality and different Mexican localities suggest the likelihood that all northern meadow frogs could not cross with their southerly-distributed lowland relatives in Mexico. The geographically extreme members of the species range have built up different adaptive gene complexes to the extent that they are incapable of producing viable hybrids in the laboratory.

It must be admitted that the probability of a Wisconsin meadow frog crossing with a Mexican frog in nature is extremely remote. Yet it is just this point which emphasizes the fact that incipient isolating mechanisms do not develop initially for the effect itself, but are simply the inevitable consequence of two populations accumulating sufficient adaptive genetic differences during a period of geographical separation. Muller (1942) was among the first to favor this concept that isolating mechanisms arise as a by-product of genetic divergence of allopatric populations. The genetic changes which arise to better adapt one population to particular environmental factors may also be instrumental in isolating to varied degrees that population from other populations which may possess different adaptive, incidentally isolating, pleiotropic alleles. In our case, genetic differentiation of the allopatric *pipiens* populations has proceeded to such a marked degree that isolation is almost complete. Presumably the genes or gene complex governing different temperature requirements of the two types of eggs also have very strong isolating effects. If the Wisconsin and Mexican *pipiens* were ever to meet in nature, natural selection would probably reinforce the isolation thru hybrid inviability by additional mechanisms which would guard against the production of hybrids and thus prevent the wastage of reproductive energy. Recently, Koopman (1950) has demonstrated experimentally an increase in the amount of reproductive isolation between two species of *Drosophila* as a result of continual artificial selection.

Of embryological interest is the demonstration that the results of hybridization between the two geographically extreme groups are very dissimilar in the reciprocal crosses. The gastrulation process is differentially affected in each type of hybrid development. In

the cross Mexican *pipiens* ♀ X Wisconsin *pipiens* ♂, the invaginative phase (internal rearrangement of groups of cells) of gastrulation is most seriously hampered, whereas in the reciprocal cross the progress of the blastopore lips over the yolk (epiboly of gastrulation) appears to be most disturbed. These early defects in the gastrular movements are responsible for the major subsequent abnormal form changes in each type of cross. In the cross Mexican *pipiens* ♀ X Wisconsin *pipiens* ♂, restriction of internal yolk movement results in the failure of the blastocoele to be completely obliterated. As the persistent blastocoele offers resistance to the involuting chorda-mesoderm, the latter is abnormally disposed in the anterior head region and this probably accounts for the subsequent microcephaly. In the reciprocal cross (Wisconsin *pipiens* ♀ X Mexican *pipiens* ♂), restriction of the epibolic spreading of the animal hemisphere results in a permanently open blastopore. The exposed yolk-plug later cytolyzes under the irregularly curved, shortened tail. Although the abnormalities in the posterior region of these hybrid embryos are traceable back to an open blastopore, the origin of macrocephaly in the anterior region is not apparent from the observations made.

As has been shown, normally fertilized eggs of Wisconsin and Mexican *pipiens* differ considerably in their rate of development. This is the most obvious physiological difference between the two eggs, and one may be tempted to propose that an asynchronous developmental rate in the hybrid egg is the factor initially responsible for the observed abnormalities. It is hazardous, however, to suggest that this may be the actual causative mechanism. The hybridization experiments were conducted at 18.9°C and 21.1°C; temperatures at which the developmental rates of Wisconsin and Mexican eggs differ. It would have proved interesting to run the hybridization experiments at 19.8°C, a temperature at which (interpolated from figure 1) the rates of development are the same. An insufficiency of frogs from Tamaulipas, Mexico frustrated efforts along this line, but it appears likely that the same types and intensity of developmental abnormalities would have been encountered if the reciprocal crosses were conducted at 19.8°C. The causative mechanism thus remains problematic. Others have attributed heterogenic hybrid abnormalities to a variety of factors: altered activity or inactivation of the sperm nucleus, disturbance of the nucleo-plasmic ratio, competition of enzymes for limited substrates, etc.

Successful interpretation of our case and others is further complicated by the demonstration that modifications in amphibian development produced by direct action of a variety of chemical and physical agents simulate in many ways inhibitions in hybrid development (see Child, 1941).

Summary

1. The geographically extreme members of *Rana pipiens* from Tamaulipas, Mexico, and Oshkosh, Wisconsin, are characterized by different embryonic rates of development and embryonic temperature tolerances, which may represent differential adaptations to the water temperatures in nature to which the eggs of each group are subjected.

2. Experimental hybrids between Wisconsin and Mexican meadow frogs show such marked embryonic abnormalities that none would survive if the respective parental populations were to become sympatric and hybridize in nature. The evolutionary tenet that the earliest indication of reproductive isolation appears as a by-product of the genetic divergence of allopatric populations is supported.

3. The types of embryonic abnormalities in the reciprocal hybrids follow definite patterns. Hybrid embryos derived from the cross Mexican ♀ X Wisconsin ♂ exhibit persistent blastocoeles, microcephaly, fused suckers and olfactory pits, reduced mouthparts, and atypical eyes. Hybrid embryos from the reverse cross, Wisconsin ♀ X Mexican ♂, possess enlarged yolk plugs, macrocephaly, overdeveloped suckers, shortened bodies, and curved tails.

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