A PRELIMINARY STUDY OF THE EFFECTS OF ADMINISTERING ETHYL ALCOHOL TO THE LEPIDOPTEROUS INSECT *SELENIA BILUNARIA*, WITH PARTICULAR REFERENCE TO THE OFFSPRING.

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I. Introductory.

My attention was first drawn to the present subject during the course of an examination of the facts published by Morgan and his pupils concerning their work on the fruit fly, *Drosophila ampelophila*. No one can study that work without being struck by the immense number of hereditable variations which have appeared, and continue to appear, in their cultures. So great and important are these when compared with the few similar mutations encountered in Nature that one is almost forced to the conclusion that in some way or other the technique employed is correlated with the observed results. Having conceived this notion, I submitted their methods to careful scrutiny but could only find one definite point in which their procedure differed from that followed by me when rearing lepidoptera or phytophagous hymenoptera or coleoptera, and that was that every individual fly used in the cultures had, at some period of its life, undergone etherisation; that fact naturally attracted suspicion. Moreover, these suspicions were strengthened when brought into conjunction with the wonderful tales one hears of the degeneracy in the offspring of alcoholic parents, as well as with the actual impairment of the young seen by Stockard in his alcoholised guinea-pigs. It thus seemed exceedingly probable that these effects were directly induced by the action of the ether on the germ cells of the parents at some very susceptible stage of their existence. Further, after careful consideration, it appeared in my view to follow as a natural corollary that if the single etherisation of the *Drosophila* flies were replaced by the continuous exposure of the organism throughout the

critical period of gametogenesis, from the earliest spermatogonial or oogonial division to the development of the perfect spermatozoon or ovum, results more important still might be obtainable. For such an experiment a lepidopterous insect, particularly a double brooded Geometer, was eminently suitable, and to carry it out a stock of the bivoltine race of *Selenia bilunaria* was secured.

Immediately after I had made these arrangements, Pearl's papers, in which he gives complete details as to his experiments on the administration of methyl and ethyl alcohols and ether to poultry, came to hand. So different were the results described there from those of Stockard, and so apparently contradictory to them were they, that it almost seemed impossible for one to harmonise them in the slightest. Stockard found that his second generation guinea-pigs were so affected that the various litters included a heavy proportion of more or less malformed weaklings: and this very nearly represents the state of affairs in similar experiments, conducted with lead salts on the same animals, by Cole, Bachhuber, Weller and others. Pearl, on the contrary, found that the progeny of alcoholised fowls was measurably superior in several important respects to the offspring of untreated individuals. Almost in complete agreement with Pearl's work were the indications of Nice's white mice, and of Elderton and Pearson's studies on the influence of parental alcoholism in human beings. The scope of my projected experiments was greatly enlarged by the possibility that they would throw some light on the seeming discrepancy between the conclusions to be drawn from the various sets of work enumerated. Furthermore, as a group of animals far removed from the Mammalia or Aves was involved, the value of the work was greatly enhanced, whether its indications went to corroborate or to contradict any or all of the data just mentioned.

The questions then I set out to answer were

(1) Can I by the continued administration of ethyl alcohol so affect the germ cells of *Selenia bilunaria* as to repeat the conditions of Morgan's *Drosophila* cultures where hereditable variations appeared in very unusual numbers?

(2) Failing this, are the progeny of parents submitted to the action of the deleterious agent significantly affected by it?

(3) If not, do the actual phenomena encountered approximate to those observed in the case of Pearl's fowls and of Cole's white mice?

(4) And generally, what effects, somatically or otherwise, has the agent used on the treated individuals or their offspring?

As will be seen below, to the first and second questions I obtained a decidedly negative answer: in the case of the third, although not precisely the same as those with which comparison was desired, my results showed a considerable approach thereto in a broad fashion. The answers to the fourth will manifest themselves as the facts of the experiments are gradually unfolded.

II. TECHNIQUE EMPLOYED.

For several reasons, some personal and some arising from the war, after a short while it was deemed best that I should restrict myself to the use of ethyl alcohol throughout the work. At first sight, the difficulties attending the administration of this compound to lepidoptera seemed insuperable, and indeed are so if one wishes to employ the agent in the liquid form; these obstacles, however, quickly vanish if one considers the ease with which insects are affected at any stage of their life-histories by the vapours of chloroform, ether, hydrocyanic acid, sulphur dioxide and the like. The only trouble remaining is to devise some means of regulating the dose and of ensuring that its incidence is long enough. Here the advantages of utilising a Geometrid moth appertaining to such a hardy genus as *Selenia* are at once realised; a considerable number of their larvae can be reared in airtight cages without the slightest ill-effects manifesting themselves. If once the use of airtight cages is granted methods of generating constant and steady supplies of alcohol vapour so that ova, larvae, and pupae are always under its action are very simply designed. After a few preliminary experiments to determine the optimum quantity to be employed, one readily finds that by saturating a small sponge periodically with definite quantities of alcohol the atmosphere within the cage is kept uniformly charged with the fumes. Thus the creatures are never at any period of their growth and development free from its possible influence either on their germ or on their somatic cells. Only one precaution is necessary, and that is to suspend the sponge so that the larvae can never come into contact with the liquid used, which, in my case, was the ordinary 90% non-methylated ethyl alcohol of the chemist.

To secure the larvae experimented with, a healthy male and female *Selenia bilunaria* were taken from a brood once inbred and paired; the female in due course laid over a hundred fertile ova. In order to avoid overcrowding as far as possible, ninety of these were divided into two random batches of forty-five, one to be submitted to the alcohol regimen,

and the other to be enclosed in exactly the same kind of cage to serve as a control and therefore minus the alcohol. The value of the controls thus used was greatly increased by the fact that they were part of the same family as the treated lot.

The treatment was commenced soon after the ova were deposited, those to be alcoholised being shut up in airtight glass-topped tin boxes with a soaked sponge, and the others put into a similar box without alcohol.

The sponge was charged twice per day, once in the early morning and once at 8 P.M. Even after hatching, during their first instar, the larvae were kept in the same tins, but as they grew larger they were removed to bigger cages, and the dose of alcohol was simultaneously increased.

After pupation the treatment was still continued; be it ever so small, respiration takes place in the pupae, gametogenesis is being completed, and therefore the germ cells are capable of being influenced at a most critical time.

Throughout their early stages, both lots were provided with exactly the same food, hawthorn (*Crataegus oxyacantha*), and at the same time. Further, since the mortality (as will be seen below) was greater amongst the "alcoholists," whenever the cages were cleaned out the number of individuals in the control cage was reduced so as to correspond with those still alive in the other; this step was, of course, taken to eliminate possible errors arising from differences in the number of cubic inches of cage room allowed to each insect.

III. The Course of the Experiment.

Although both sections of ova produced their full complement of larvae about May 4th, the effects of the alcohol were very soon visible when once the larvae commenced to feed, for almost immediately the mortality rate amongst the treated individuals became very great, no less than fourteen ($=31{\cdot}1\%$) dying in the first instar. On the other hand, only two ($=4{\cdot}4\%$) succumbed in the untreated controls—a number showing no sensible divergence from one's usual losses with first stage larvae. At this point, as shown in Table I, the controls were reduced to 31 so that once again the cages contained equal numbers. With the weeding out of these presumably very susceptible specimens the death rate in the intervals between the changing of the food and the cleansing of the cage slowed up, 13%, $14{\cdot}9\%$ and $17{\cdot}3\%$ being the

respective losses of the "alcoholists." The natural deaths amongst the untreated examples retained to act as controls were absolutely zero. However, as will be seen, one death is noted; this single death, as well as one of those marked with an asterisk in the other column, was due to the accidental crushing of the larvae by the twigs of the food plant.

TABLE I.

Date	Number of individuals treated with alcohol	Number of deaths amongst the treated larvae	Number of controls	Number of deaths amongst controls	Control reduced to
4/5/18	45	—	45	—	—
8/5/18	31	14	43	2	31
14/5/18	27	4	31	—	27
20/5/18	23	4	27	—	23
28/5/18	18	5*	22	1*	18
3/6/18 Pupation commences	10	8	18	—	—

One must be careful to explain that the two groups were not differentiated by the death rate alone; such a supposition does not agree with the facts. On May 18th, when the bulk of the controls cast their first skin, they did so a day and a half ahead of the most precocious member of the other lot, and the advantage thus gained was never lost; it was, on the contrary, increased so that on May 28th they were three days ahead, and when pupation commenced on June 3rd they span their cocoons six days earlier than their "drunken" brothers and sisters. In reality this latter interval should be reduced to five days because on May 30th, when all of the treated larvae had entered their last larval instar, the amount of alcohol administered was so excessive that all save three were stupefied and lay as if dead for 34 hours. The sponge was saturated at 6.30 A.M. and the larvae affected rendered unconscious by 7 A.M. When examined next day at 7 A.M., three had recovered, the rest following suit before 5 P.M. the same day.

Although possibly due to the deleterious effects of the ethyl alcohol on weaker and therefore smaller larvae with their consequent destruction, the retardation in growth was apparently linked up to some extent with the fact that the individuals exhibiting it attained a greater mean size than the majority of the controls did. In consequence, they produced correspondingly heavier pupae and larger imagines, thereby necessitating a longer period to reach their full growth.

Of the alcoholised caterpillars, ten, comprising seven males and three females, succeeded in spinning their cocoons and pupating therein; of the others, eighteen (including ten males and eight females) did likewise. The difference in sex ratios, in view of the small numbers

involved, has, in all probability, no definite import, although it is conceivable that the exaggeration of the disparity in the case of the treated lot points to some superior resistive powers on the part of the female zygotes to the effects of alcohol vapour.

Emergence commenced with the appearance of a control male on June 15th, to be followed at intervals by others until every single pupa had yielded its imago. The other group began to put in an appearance on June 18th, but of the ten pupae only seven, two males and five females, emerged. The remainder died shortly after pupation whilst the alcoholisation was yet being continued.

Thirty per cent. therefore died as pupae. Of those emerging two, one of each sex, were cripples. The male was a large strong insect and owed its crippling to accidental injury; the female, on the other hand, was a weakling and did not succeed in disentangling itself from the pupal integument. Had it not been freed artificially it would, undoubtedly, have perished within the pupal envelope.

As the imagines came out they were caged up in three separate muslin cages, the controls being paired *inter se*, and reciprocal pairings being made between them and opposite sexes of the "alcoholists." Owing to the fact that I never had a treated male and female out together, the fourth possible pairing, that between a male and a female treated example, proved unobtainable. When all of the insects had died, they were removed from the pairing cases and carefully measured with results seen in the appended table. It is perfectly evident that, in

TABLE II.

Wing Expanse	Alcoholised Individuals		Controls	
	Number of Males	Number of Females	Number of Males	Number of Females
34 mm.	—	—	3	—
35 mm.	—	—	2	—
36 mm.	1	—	3	5
37 mm.	—	1	—	2
38 mm.	—	1	—	1
39 mm.	—	2	—	2
Cripples	1	1	—	—
Total	1+1	4+1	8	10
Average wing expanse	36 mm.	38·25 mm.	35 mm.	37 mm.

both sexes, the insects which have been placed under the action of ethyl alcohol fumes during their growth and development periods are distinctly larger in size. In spite of the low numbers concerned, I feel certain that this is no chance effect, more especially when this superiority

in size over their untreated relatives is just what Pearl noted in his fowls.

In all cases the copulations proved successful, and an abundance of fertile eggs was laid. As a matter of fact every egg in the cages changed colour and thereby displayed its fertility. The exact total of those deposited in the cages by fertilised females, and in chip boxes by virgin females, is supplied with other essential facts in Table III.

TABLE III.

	Alcoholised Females		Control Females	
	Number of Individuals	Ova laid	Number of Individuals	Ova laid
Paired with control ♂♂ ...	2	248	5	603
Paired with alcoholised ♂♂	—	—	2	254
Not paired	2	274	3	386
Totals	4	522	10	1243
Average number of ova per female	-	130·5	—	124·3

Again as tested by the average number of ova per female, the untreated individuals were distinctly inferior to those which had undergone treatment with alcoholic fumes. Nor did the matter rest there; although every egg was fertile not every one hatched, and there were marked differences in the number of zygotes which perished in the shell, the mortality rate being obviously less when a treated imago figured as a parent. As with other statistical data, this is best set out in tabular form so that the precise values may be comprehended at a glance.

TABLE IV.

Pairing	Number of ova laid	Number which died in the shell	Percentage dying in the shell
Control ♂♂ × Control ♀♀ ...	603	47	7·8
Control ♂♂ × Alcoholised ♀♀ ...	248	5	2·01
Alcoholised ♂♂ × Control ♀♀ ...	254	7	2·7

Although the ova from the controls were laid in the four days following June 16th, those from the mating of an alcoholised male and a control female in the four days after June 19th, and those from the reciprocal cross after June 21st, the ova hatched in the reverse order; those from the last-named pairing emerging on July 1st, 2nd, and 3rd, those from the second lot on July 2nd, 3rd, and 4th, and those from the untreated controls spreading themselves out over the period between July 8th and July 14th, both days being included. Here we first see indicated that acceleration in development manifested by the offspring of treated parents at every stage.

As before, instead of rearing all the larvae, a random sample of fifty ova was chosen from each batch, and the resulting larvae fed up on hawthorn, the food plant of the parents. Unlike them, however, the three sets were reared in well-ventilated and well-lighted cages such as are employed in normal breeding operations. In the first four instars no deaths occurred in the crossings in which treated insects took part; even in the inbreeding of the controls the loss was small, the deaths in each skin being one, three, none, and four respectively.

When, however, the first larvae were ready to spin, these being derived from a pairing between a treated male and an untreated female, there were left fifty of that cross, forty-six of the reverse brood and thirty from the controls. In view of the fact that very great differences in speed of feeding up were now most strikingly displayed, it will be best to show the numbers spinning up and pupating each day in the form of a tabular diary. In this table (Table V) records are set out daily until August 22nd, when all of the earliest lot had spun, and for two day intervals subsequent to that.

TABLE V.

Date	Alcoholised Males × Control Females		Control Males × Alcoholised Females		Control Males × Control Females	
	Spun	Pupated	Spun	Pupated	Spun	Pupated
August 8th	3	—	—	—	—	—
August 9th	5	1	—	—	—	—
August 10th	11	2	5	—	—	—
August 11th	15	5	7	3	—	—
August 12th	21	12	10	6	1	—
August 13th	25	15	11	8	1	—
August 14th	31	21	13	10	2	1
August 15th	37	26	17	11	4	1
August 16th	40	32	18	13	6	2
August 17th	42	37	20	17	7	4
August 18th	44	40	22	18	9	5
August 19th	46	42	25	20	10	8
August 20th	50	44	28	21	14	9
August 21st	50	46	28	24	15	10
August 22nd	50	50	30	27	17	14
August 23rd—25th	50	50	30	28	19	17
August 25th—27th	50	50	32	30	23	20
August 27th—29th	50	50	32	30	24	21
August 29th—31st	50	50	36	32	25	21
August 31st—September 2nd	50	50	37	32	25	24
September 2nd—4th	50	50	41	36	25	25
September 4th—6th	50	50	41	41	28	26
September 6th—8th	50	50	41	41	28	27

From the above figures it is very clear that the total loss in the cross between an alcoholised male and a control female is zero, that in the reciprocal cross is nine (= 18 %), and amongst the controls twenty-

three (= 46%). This result is the more significant when one recollects that all three lots were descended from the same great-grandparents and grandparents and had thus been twice inbred—a fact explaining the low vitality of the controls which the administration of alcohol to the parents had apparently surmounted in the case of the others.

Nor was the superiority where the parentage included an alcoholised individual confined solely to the low death rate; it was exhibited in the general rapidity of feeding up and growth as can readily be gleaned from the facts and data given. Even more remarkable was the fact that, despite the greater rate of growth, the members of the same two broods attained a much greater size in both sexes. To enable this to be grasped with facility use is once more made of a table. Profiting from my experience gained with their parents, when cripples were not available for measurement, in compiling Table VI, instead of taking the imaginal wing expanse I extracted the pupae from their cocoons, weighed them and utilised the pupal weights which appear, classified according to brood and sex, below.

TABLE VI.

	Males			Females		
Brood	Number of Individuals	Weight	Average Weight	Number of Individuals	Weight	Average Weight
Alcoholised ♂ ♂ × Control ♀ ♀	23	3·79 grms	·165 grm	27	5·25 grms	·194 grm
Control ♂ ♂ × Alcoholised ♀ ♀	18	2·55 grms	·142 grm	23	4·07 grms	·177 grm
Control ♂ ♂ × Control ♀ ♀	12	1·44 grms	·12 grm	15	2·02 grms	·135 grm

The insects began to come out on August 24th when a male proceeding from the pairings in which the alcoholised male took part appeared. Others followed by degrees until August 27th when four males and one female had been bred. On that date two females representing the reverse cross joined company. The first control, a male, only emerged on September 1st when the other two batches were nearly all out: in fact, two-thirds of the treated male × untreated female lot and one-half of the other were already on the setting boards. Nevertheless, within the ensuing seven days, every insect had been reared save for one control which had died in the cocoon as an unchanged larva.

Just as one might anticipate from their greater pupal weight, the imagines from the alcoholised series were much the larger insects in both sexes. In addition to this, they displayed their robustness in a very

noteworthy and unexpected way. I mentioned above that the race of *Selenia bilunaria* with which we are concerned was double brooded, but I did not then indicate that it was seasonally dimorphic; such, nevertheless, is the case. The spring brood consists of larger, heavily and richly pigmented individuals, whilst the summer[1] brood is smaller and very weakly provided with pigment when compared with its spring relatives. This difference is caused directly by the longer growth period of normal autumnal larvae (which produce the spring imagines) lending itself to the production of larger, sturdier pupae. In spite of the circumstance that the two broods including a treated example in their parentage actually fed up more quickly than the controls, they exhibit in most of their members a facies exceedingly close to that of an ordinary spring brood insect for they possess all its depth and richness of coloration—an occurrence depending immediately on their superior size and vitality. The controls, on the contrary, are smallish specimens differing in no respect from the washed out looking July brood which Haworth called *juliaria*.

In the progeny of Stockard's alcoholised guinea-pigs one of the most striking things was the development of individuals malformed in respect to various organs, but more especially in the eyes. In the case of these insects now being discussed, no such abnormalities presented themselves; all of the imagines, no matter what their parentage, were equally perfect in all of their organs.

The sex ratios in all cases call for no comment although in all, exactly as in the broods from which their parents were derived, an excess of females appears.

IV. Discussion of the Results.

To summarise the results we may state:

(1) The offspring of treated parents neither included monstrosities nor displayed even slight abnormalities.

(2) No new hereditable variations occurred.

(3) The survivors of the treated batch grew, on the average, decidedly larger than their untreated relatives. This may have arisen simply from the elimination of the weaker individuals by the deleterious agent employed, or it may have been caused by some physiological action of the ethyl alcohol. In any case it agrees with Pearl's experience with

[1] Should, by chance, three broods appear in one season the third resembles, in every respect, the second or normal summer brood.

his poultry; since his mortality rate was negligible, in all probability we have to look to both of the suggested causes for a correct explanation in this instance.

(4) The progeny of the treated batches was greatly superior in several respects to that of the untreated controls.

(5) The offspring from the cross between a treated male and an untreated female was superior to that of the reverse mating to much the same degree as the latter was better than the controls.

(6) The superiority of the broods originating with treated parents was exhibited in:

(*a*) The smaller percentage of embryos perishing in the shell, the exact figures being 2·01 °/₀ and 2·9 °/₀ against 7·8 °/₀.

(*b*) The quicker development of the embryo.

(*c*) The speedier rate of feeding up in the larva.

(*d*) The lower rate of larva mortality, 0 °/₀, and 18 °/₀ against 46 °/₀.

(*e*) The greater mean weight of the male pupa.

(*f*) The greater mean weight of the female pupa.

(*g*) The quicker development of the imago and its earlier appearance.

(*h*) Its richer pigmentation.

(7) In sex ratio and in the percentage of fertile eggs no differences were perceptible.

Except, therefore, in the percentage of fertile eggs and in characters only capable of manifestation in the lepidoptera, my data in the main tend to point in the same direction as those of Pearl.

To explain the results I think we have to look to the cumulative effects of selection of two different types acting at different stages: (I) the first of these acts on the parental zygotes when the alcohol weeds out the weaker insects and preserves the stronger to perpetuate their race; (II) the second works directly on the germ cells of these selected, stronger, and sturdier survivors.

The first point needs little or no elaboration as it is almost self-explanatory; still perhaps a few words are necessary. As only insects superior in size and vigour survived the action of the alcohol to act as parents it is a fair inference that their gametes would be of a superior type. Of necessity, then, zygotes into whose composition these gametes enter would manifest greater strength and vitality. Thus if the qualities in which the broods from alcoholised parents excel depend in part at least on parental excellence then such broods would

commence life with a weighty advantage in that respect over the progeny of untreated lots which have undergone no similar process of selection.

The second part demands more consideration. Throughout the lives of the parent insects their germ cells have been constantly liable to injury from the alcohol vapour. Now, as Pearl suggested, almost certainly the germ cells will not be alike in resistive powers, and therefore the alcohol will not affect all in a similar manner. Merely considering the germ cells as acted on by a medium dose of alcohol, there are three possibilities: (1) that weaker germ cells can be destroyed or rendered wholly ineffective; (2) that more vigorous ones can be weakened; (3) that germ cells of the Grade I type can be so refractory as to be quite unaffected. Clearly, too, by increasing the dose of alcohol we could progressively eliminate all three classes. Supposing that the amount employed in the present experiment was just sufficient to destroy the feebler and medium members of the germ cell population, what would that lead to? It would only leave the most superior to continue the race. If, as seems undoubtedly the case, an "A 1" germ cell population guarantees "A 1" zygotes, then the subsequent generation should all be "Grade 1." And this exactly fits in with the insects reared from the cross between a treated male and an untreated female. Every single imago of this parentage was vastly superior to all controls. Therefore selection, in the first place of first quality parents producing the finest type of germ cells, followed by intense selection amongst these chosen germ cells themselves, affords an adequate explanation of the circumstances of this brood.

If, however, the weaker cells alone are inactivated, and the stronger and medium left, the former absolutely uninjured and the latter affected in varying degrees, then the zygotes resulting from gametes so differentiated would be of two grades: (1) composed of sturdy individuals much the same as what we have just considered in the case of the previous mating; (2) including distinctly less vigorous examples. This agreed precisely with the state of affairs in the insects raised from the pairing of an untreated control male and an alcoholised female.

From the foregoing it will be perfectly obvious that, despite the fact that both alcoholised males and females received the same dose of ethyl alcohol and for the same time, the conditions of the broods from reciprocal crosses in which these insects took part were utterly dissimilar. It therefore follows that, as far as these insects are concerned, the gametes of the female are much less susceptible to

alcoholic selection than those of the male—which is entirely in accord with one's expectations after giving due attention to the protection afforded to the gametes of the female for such a prolonged period of their existence.

That the whole of the zygotes yielded by the mating of an alcoholised male and a control female were substantially alike, and that those from the reverse pairing were not so, was actually capable of proof. When exactly one-half of the first-named lot had pupated they were weighed, as were also the representatives of the other lot then in pupa. A similar course was then adopted when the other half had changed. It was then discovered that, whereas the two halves of the former agreed in the mean weights of the male and female pupae, the second (as well as the third) portion of the other cross was clearly inferior to the first as will be readily perceived from the following table:

TABLE VII.

	Alcoholised Males × Control Females		Control Males × Alcoholised Females	
	Average Male Weight	Average Female Weight	Average Male Weight	Average Female Weight
First Portion, August 15th	·164 grm	·191 grm	·163 grm	·186 grm
Second Portion, August 23rd	·195 grm	·198 grm	·141 grm	·177 grm
Third Portion, September 6th ...	All emerged by Aug. 23rd	All emerged by Aug. 23rd	·126 grm	·171 grm

If this hypothesis is in very truth the scheme that forms the groundwork of the observed facts then my insects, when the requisite allowance is made for the additional phenomenon of parental selection, fall in line with Pearl's poultry and not with Stockard's guinea-pigs, for in the latter the destruction and injury of the germ cells of treated parents had proceeded much further than in the germ cells of my *Selenia bilunaria* males. This, too, serves to demonstrate why neither Pearl nor I reared malformed progeny while Stockard did so.

In conclusion, only one more point needs explaining, and that is the great feebleness of the controls. This is quite consistent with one's expectations. The genus *Selenia*, like many (but not all) lepidopterous genera, is extremely impatient of inbreeding and soon dwindles in size, productivity, and vigour under its influence. That parental and gametic selection was able to counteract this only emphasises further the lessons of the experiment, for it must not be forgotten that controls and "alcoholists" alike had the same great-grandparents and grandparents, and that, necessarily, their immediate parents were brothers and sisters.

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