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WITHDRAWN

AN EVALUATION OF AN EXPERIMENTAL MODEL FOR SYMPATRIC SPECIATION

by

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ABSTRACT

With slight modifications, an attempt was made to repeat the work of Pimentel, Smith and Soans (1967) whose experimental results suggested that disruptive selection on two partially isolated populations of *Musca domestica* could result in sympatric speciation. The two strains of flies were selected for preference for two different ovipositional attractants with approximately 90% selection pressure for correct choice.

The experiment was pursued through four generations and a number of factors found which complicate interpretation. Because very few flies selected the ovipositional attractant "correctly" at first, the number of flies being used as parents for each generation were very low, resulting in a high degree of inbreeding, and the possibility of gene drift. Numbers of eggs laid, pupal hatch times and migration rates all showed a great variability between the two strains, with gene drift as a possible cause. In addition, freshly laid eggs were found to act as ovipositional attractants, introducing a further source of error into the breeding system.

These are problems that need to be overcome before this type of experiment can be used as evidence for sympatric speciation.

INTRODUCTION

In 1967, Pimentel, Smith and Soans published a paper on the effects of disruptive selection on two partially isolated populations of *Musca domestica*, in an attempt to produce a population model of sympatric speciation. Briefly, they selected two races of flies for ovipositional preference for either fishmeal or banana peel with approximately 90% selection pressure for correct choice. This was achieved by offering flies one "correct"* choice out of ten (the other nine being the correct attractant for the other strain) and allowing only those making the "correct" choice to contribute to the next generation in that cage. The ten phials of attractant for either strain were placed in two large cages separated from each other by two smaller cages. The smaller cages aided

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*For all selection work flies laying eggs in the one phial of attractant selected for in each cage were regarded as having chosen "correctly". Use of this term "correct choice" is for expedience of description and should not be interpreted as being indicative of effectiveness of selection.

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the detection of migration of flies between the larger cages. All cages were connected by nine inch lengths of clear perspex tubing, one inch in diameter. The flies of one strain were introduced to one end cage, those of the second strain to the other, at the beginning of each generation. Flies of each strain were distinguished by colour markings and thus frequency of migration was easily determined.

Assuming that fishmeal flies evolved a preference for fishmeal and banana flies a preference for banana, hybrids would be at a disadvantage in either population and therefore would be selectively eliminated. Since significant differences for ovipositional preference were noted in both strains, Pimentel *et al* felt that sympatric speciation was in fact occurring. However they did not look for reproductive isolation which would have given conclusive evidence of sympatric speciation.

The initial aim of this study was to follow this previous work as closely as possible, culminating the work with suitable reproductive isolation tests. However attempts to follow the experimental methods of Pimentel *et al* met with failure on several counts and never got as far as reproductive isolation tests. This paper is a brief review of these problems. It is felt that the problems create difficulties in presenting this type of work as evidence for sympatric speciation.

METHOD

Origin of Flies

Flies were caught in January 1969 at the Wilton Tip, Wellington, using a butterfly net.

Variations in Method

It must be mentioned at the outset that the method of Pimental *et al* was not adhered to in exact detail. Fishmeal and banana peel were used as attractants for one experimental trial, but an overwhelming preference for fishmeal caused extinction of the banana flies after only one generation of selection. (A summary of the results of this work can be found in Appendix I). After a number of trials with a cage of approximately 500 flies and a variety of potential attractants, ammonium carbonate and amyl alcohol were chosen as attractants. (Ammonium carbonate was dissolved in water at concentrations of 100gm per 100cc cold water and amyl alcohol was diluted to 0.04 strength in a fine suspension with water). For egg-laying, several drops of chemical were placed on a chopped-up cube of yeast-agar-milk powder medium. This gave a physical structure of cracks and crevasses conducive to egg-laying. This substitution of chemicals may have affected the results in that flies were given only two bases for choice, smell and taste, whereas with organic ovipositional attractants there are four bases for choice—smell, taste, structure and sight.

Two other modifications of experimental method were employed. Flies were not marked with model aeroplane paint on the thorax but had a small colour marking applied to one wing tip. The paint used was a

mixture of clear nail varnish and a fine luminous powder. Counting was done with the aid of an ultra-violet lamp (Pal, 1947). This did not appear to affect the behaviour of the flies and avoided the necessity of daily anaesthetisation with carbon dioxide, as flies could be counted in the cages.

To get a clearer idea of trends in selection, different generations of flies were kept separate. Once the females had completed egg-laying, flies of one generation were destroyed and the cages cleaned in preparation for the flies of the next generation. Flies were sexed every 24 hours during hatching and only young virgins were used in the experimental cages. It was felt that this was important as older flies, even if virgins from early sexing, would be more likely to mate immediately on entering the experimental system, probably to a member of their own strain. For each separate generation each strain was limited to 100 males and 100 females, making a total of 400 flies in the system. In all other details the experimental method of Pimentel *et al* was adhered to. The final experiment ran through only four generations before having to be discontinued through lack of time.

Parameters Recorded

(i) number of eggs laid each day in each generation per phial.

(ii) percentages of eggs laid correctly per day and per generation.

(iii) average percentage migration per strain per generation.

Additional Tests

(a) *Testing equal amounts of each attractant*

At the end of the experimental work, a test was run on the 200 female flies that would have been selected for the fifth generation. When they were fully developed and ready to lay eggs, the flies were given the choice of equal amounts of each attractant to determine whether or not they chose the correct attractant for their strain in a higher percentage than the attractant of the opposite strain. Each strain was given three egg-laying phials of each attractant since it was felt that this would reduce the chances that an accidental heavy laying in one phial would distort the results (this distortion effect is dealt with in the discussion). The experiment was repeated on four successive days.

(b) *Eggs as attractants*

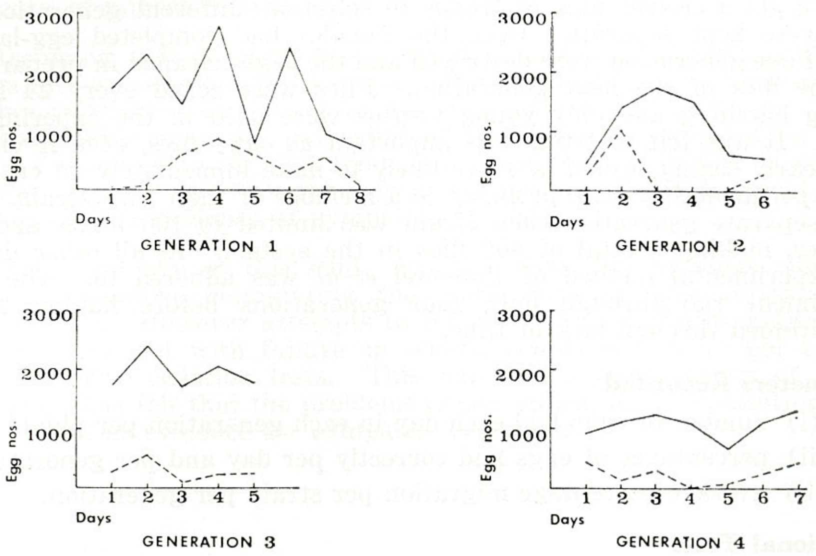
This test was performed in an attempt to assess the possibility that freshly laid eggs might act as ovipositional attractants. Sample populations of 200 females flies were offered a choice of two egg laying phials, one having the egg laying medium scattered with freshly laid eggs, the other being untreated egg laying medium. Numbers of eggs used as "attractant" were counted before use in order that they would not weight the results of the experiment.

(c) *Strength of chemical vs egg attractants*

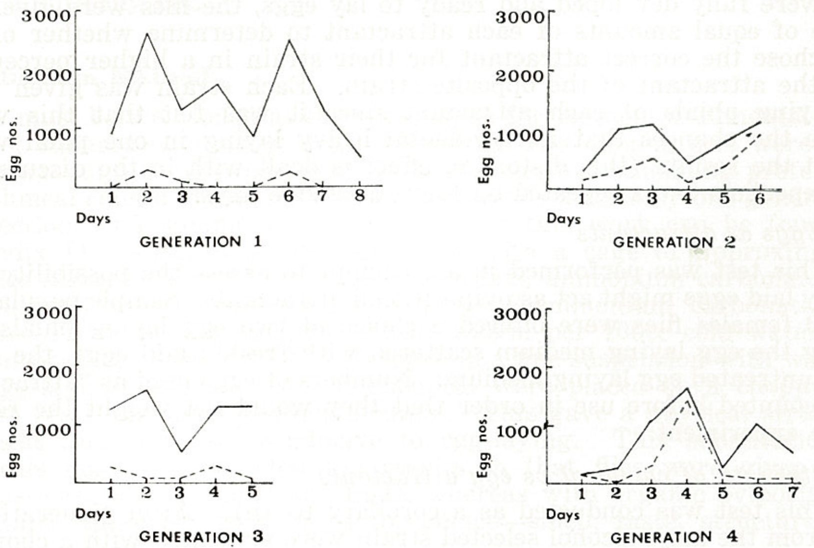
This test was conducted as a corollary to (b). After Generation 4, flies from the amyl alcohol selected strain were presented with a choice of two egg laying phials, one containing egg laying medium and amyl alcohol, the other egg laying medium and eggs. Similarly the ammonium carbonate selected flies were presented with two choices, either ammonium carbonate on egg laying medium or egg laying medium and eggs.

Fig. 1. TOTAL AND CORRECT NUMBERS OF EGGS LAID DAILY BY EACH STRAIN.

a) AMYL ALCOHOL STRAIN



b) AMMONIUM CARBONATE STRAIN



KEY for Figs. 1a and 1b.

——— = Total eggs laid

- - - = No. of eggs laid correctly

Fig. 2a.

DAILY PERCENTAGE OF EGGS LAID CORRECTLY IN EACH CAGE.

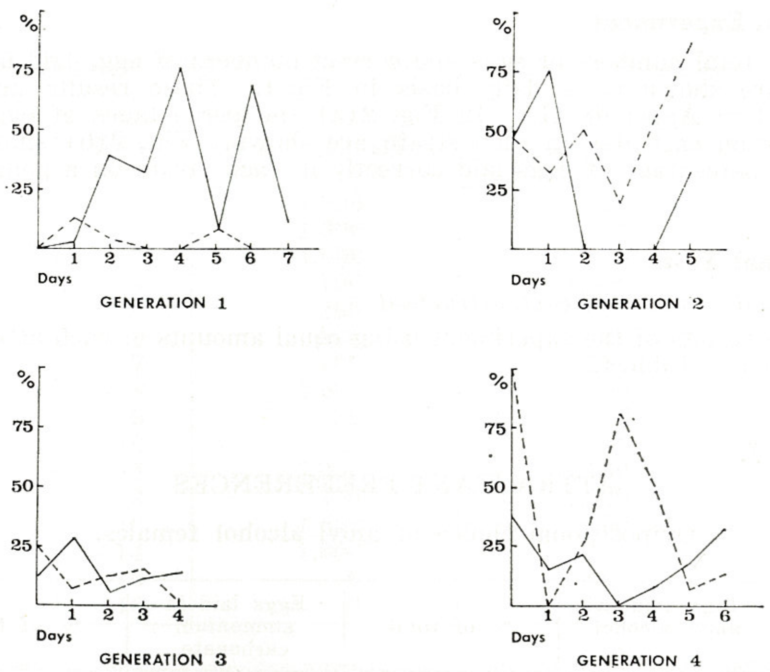
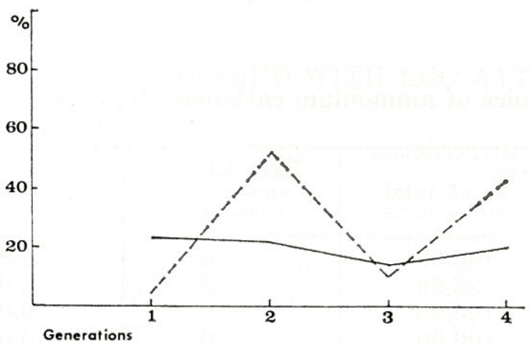


Fig. 2b.

AVERAGE PERCENTAGE OF EGGS CORRECTLY LAID IN EACH CAGE.



KEY for Figs. 2a and 2b.

——— = Amyl alcohol strain

- - - - = Ammonium carbonate strain

RESULTS

Selection Experiment

The total numbers of eggs and correct numbers of eggs laid by each strain are shown on a daily basis in Fig 1. These results are also tabulated in Appendix II. In Fig. 2(a) the percentages of eggs laid correctly on each day in each strain are shown. Fig. 2(b) shows the average percentage of eggs laid correctly in each strain on a generation basis.

Additional Tests

(a) *Equal amounts of each attractant*

The results of the experiment using equal amounts of each attractant are shown in Table I.

TABLE I.

ATTRACTANT PREFERENCES

Ovipositional choice of amyl alcohol females.

Day	Eggs laid in amyl alcohol	% of total	Eggs laid in ammonium carbonate	% of total
1	163	56.75	128	43.25
2	1,227	60.18	812	39.82
3	95	100.00	0	0.00
4	196	53.70	169	43.06

Ovipositional choice of ammonium carbonate females.

Day	Eggs laid in amyl alcohol	% of total	Eggs laid in ammonium carbonate	% of total
1	34	100.00	0	0.00
2	1,129	96.99	35	3.01
3	278	100.00	0	0.00
4	249	100.00	0	0.00

(b) *Egg attractant effects*

From a total of 16 trials, 12 showed a significantly greater percentage of eggs laid in the phial containing the "egg attractant".* The results are summarised in Table II.

TABLE II.

ATTRACTION EFFECTS OF NEWLY LAID EGGS†

TRIAL No.	MEDIA and EGGS	CONTROL
1	1,000	20
2	1,000	0
3	1,000	20
4	100	1,000
5	200	20
6	500	20
7	100	500
8	1,000	500
9	50	0
10	50	0
11	1,000	100
12	100	1,000
13	1,000	500
14	1,000	100
15	0	1,000
16	0	1,000

†N.B.: These results are rounded numbers, based on volume of eggs, not counts of individual eggs.

(c) *Strength of chemical vs egg attractants*

The results of this experiment are listed in Table III.

TABLE III.

CHEMICAL COMPARED WITH EGG ATTRACTANT EFFECTS

AMYL ALCOHOL STRAIN		AMMONIUM CARBONATE STRAIN	
Amly alcohol	Eggs§	Ammonium carbonate	Eggs
0	200	100	50
200	50	50	0
500	50	500	50

§N.B.: These results are rounded numbers, based on volume of eggs, not counts of individual eggs.

*Wherever the term "significance" is used in discussing the results Chi square tests have been employed and differences have been referred to as highly significant at the 1% level.

DISCUSSION

Effectiveness of Selection

The results give very little indication that selection increased the efficiency of ovipositional choice. If selection had been successful there should have been an increase in correct laying relative to the total number of eggs laid. The results contain no indication of such a trend.

Perhaps the enormous variability of results has masked any trend that may have been present. Day to day fluctuations were large as Fig. 1 clearly shows. Furthermore a higher total number of eggs was laid by both strains in Generation 1 than in any other generation, with a very large amount of variance between the totals for the four generations ($S_2 = 41{,}977{,}318.66$). The percentages of eggs laid correctly by each strain each day were still fluctuating as markedly in Generation 4 as in any other generation. As shown in Fig. 2 there is no detectable daily or generational relationship between the percentages laid in either strain.

In the parental selection generation one would expect that approximately equal amounts of eggs would be deposited in all ten phials, and thus the one phial selected for in each population would contain approximately 10% of the eggs laid. This was not observed. The average percentage of eggs laid correctly in the amyl alcohol population in the first generation of selection (23.46%) was more than double the expected 10%, while the average percentage of eggs laid correctly in the ammonium carbonate population (4.67%) was only half that expected. By the fourth generation of selection the percentage of amyl alcohol population eggs laid correctly was actually less than in the first generation, dropping to 19.59%. At the same time the percentage of eggs laid correctly in the ammonium carbonate population rose to 42.96% in Generation 4. The overall results do not suggest that selection for correct ovipositional choice was successful in either strain.

If no variation in preference occurred the number of eggs laid correctly should tend to correlate with the total number laid, as in Generations 2 and 4 of the ammonium carbonate strain, and Generations 3 and 4 of the amyl alcohol strain. However, correlation coefficients for correctly laid, as compared with total numbers of eggs, all showed no significant relationship between the two except for Generation 4 of the ammonium carbonate strain, where $r = 0.9083$, being highly significant at the 0.1% level. While there is no significant correlation for Generation 4 of the amyl alcohol strain, the correlation coefficient ($r = 0.4184$) approaches significance more closely than those for any other generation of either strain. This tends to suggest that by Generation 4 there is beginning to be a stabilisation between total numbers and correct numbers of eggs laid.

Testing Equal Amounts of Each Attractant

A very interesting difference was obtained when females bred from the last generation of the selected strains were given a choice of equal amounts of each attractant. The females from Generation 4 of the amyl alcohol strain placed 60.32% of their eggs in amyl alcohol and 39.68% in ammonium carbonate. This result is significantly different from a 50% random choice and does seem to indicate that amyl alcohol females prefer to lay their eggs in amyl alcohol. However, the ammonium carbonate females also preferred to lay their eggs in amyl alcohol, placing only 2.03% of their eggs in ammonium carbonate, and 97.97% in amyl alcohol. Yet in the selection experiment, the ammonium carbonate strain tended to a more correct choice than the amyl alcohol strain. (In Generation 4 ammonium carbonate flies chose 42.96% correctly, and amyl alcohol flies 19.59% correctly).

Other Differences Between the Two Populations

While selection was not successful in the desired direction, it was not without effect on the two populations of flies. The first generation in both cages tended to show a two day cycle of peak and trough in egg laying which was never repeated once selection began. Some physiological pattern in egg laying may have been interrupted by the artificially imposed selection.

Furthermore, some dissimilarities did arise between the two populations during the course of selection. The ammonium carbonate strain, for example, suffered a marked reduction in the number of eggs laid. Since the two strains experienced identical conditions at any one time, and the populations were of identical size, it can be assumed that the two populations should lay approximately equal numbers of eggs in any generation. This did not happen. In every generation the amyl alcohol strain laid more eggs than the ammonium carbonate strain, the difference becoming larger as the experiment progressed, with the exception of the last generation. In successive order of generations, amyl alcohol flies laid 1,216, 1,671, 3,175 and 2,519 more eggs than the ammonium carbonate flies. These differences are highly significant.

Gene Drift — Possible Explanation of the Variability

These differences between the two strains could be explained in terms of the effect of inbreeding and gene drift on the flies. With the very heavy selection pressures employed, it is quite conceivable that very low numbers of the total population actually form the parents of the next generation. Since any one female can lay up to 150 eggs at a time (West, 1951) it is quite possible that all the eggs correctly selected in one day for one strain could have come from either one or very few flies.

Differences Between Generations That Suggest Gene Drift

The two strains exhibited great variability in pupal emergence times. In Generation 3 for example, amyl alcohol flies started emerging two days before the ammonium carbonate flies. Yet in the next generation, the ammonium carbonate flies began emerging several days ahead of the amyl alcohol flies. Here again, a feasible explanation would seem to be that the bulk of selected flies came from one, or very few females, and thus, by chance, were selected for early or late emerging. The differences in emergence times cannot be explained by temperature differences during incubation of the pupae, because all pupae were reared in comparable temperatures.

Frequency of migration for the amyl alcohol flies was lower in every generation except the third (results of migration counts for both strains are given as percentages in Appendix III). This could be because of an inbreeding effect resulting in less active amyl alcohol flies.

The main problem would seem to lie in determining just how much inbreeding and gene drift are present in an experiment of this type. Inbreeding poses practical problems in affecting the viability of flies. Gene drift seems to have caused marked variability between the two strains. Furthermore, there appears to be no way of controlling the factors accidentally bred into the populations in this way. If a population that appears so well adapted can change so drastically over the course of one generation (see second section of Discussion) it would seem that gene drift can easily outweigh the effects of selection. This problem needs to be investigated, and the experimental procedure adjusted to eliminate gene drift if possible, before the method can be used efficiently.

A Further Practical Problem — Egg Attractant Effects

Early in the experiment it was noted that freshly laid eggs tend to attract other females to lay their eggs in the same place. This gives a strong clumping effect which Pimentel *et al (loc. cit.)* mention only briefly in the appendix to their paper. Yet should one fly lay eggs wrongly, if those eggs then attract more females to deposit their eggs in the same place, a misleading distortion of results could conceivably occur.

The experiment on egg attraction effects (see Method) was designed to test this possibility. In twelve out of sixteen trials, phials containing egg "attractant" were chosen in a significantly greater percentage than phials containing only normal egg laying medium.

While these results are not completely conclusive, they do suggest that this egg attraction effect may introduce a source of error into the breeding system, which would be difficult to control. It is pointed out in order that its effects might be weighed against the variability of results.

Nevertheless, selection should favour those flies which are attracted more strongly by the chemical attractant than by other laid eggs. If this is so, less flies should fall into the trap of being attracted to an incorrect phial, resulting perhaps in a decrease in the number of phials incorrectly selected for in each generation. Unfortunately, this trend was not detected in the results. In the amyl alcohol population, the average number of phials incorrectly selected for out of nine were 5.1, 4.0, 5.3 and 4.0 for Generations 1 to 4, whilst in the ammonium carbonate populations 5.6, 2.0, 3.8 and 2.0 phials were incorrectly selected on the average in each generation.

The experiment using phials with egg or chemical attractants (see Method) was devised in an attempt to clear up this point. In five out of six trials, more eggs were laid in the phials with chemical attractant. This result tends to suggest that selection has favoured attraction to chemicals over that to eggs by this stage of the experiment. Since the problem was not anticipated, no comparable experiment was run on the parental stocks and hence no comparison can be made.

CONCLUSIONS

The experiment gives no indication of directional selection for correct ovipositional choice. Directional selection might have evolved if selection had been continued for longer, but it would appear that there are many problems which need to be ironed out before this type of experiment is held up as evidence for the sympatric speciation process.

A high degree of inbreeding and gene drift in the experimental

system is suggested by the great range of variability between strains and generations in such diverse factors as egg numbers, migration rates and pupal hatch times.

The results of these processes could well mask any positive results in the selection experiment, and hence inbreeding and gene drift should be eliminated so that the experimental system can function in an unambiguous fashion.

Further confusion in selection results is indicated by the fact that freshly laid eggs can themselves be attractants. A full investigation of this possibility seems warranted.

If these problems can be eliminated, the experimental system devised by Pimentel *et al* could provide a valuable tool in research on the nature of sympatric speciation.

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APPENDIX

I. *Results of experiment with banana and fishmeal*

Days of selection	1	2	3	4	5	6	7	
No. of eggs correctly laid in banana	0	6	0	5	57	21	0	Parents
No. of eggs correctly laid in fishmeal	47	85	75	69	53	12	30	
No. of eggs correctly laid in banana	0	0	*					Generation 1
No. of eggs correctly laid in fishmeal	75	42						

*At this point no egg numbers were counted for three days although phials were maintained. No eggs were laid correctly on banana during this period.

II. (a) *Correct and total numbers of eggs laid in the ammonium carbonate strain during the selection experiment*

	Days	1	2	3	4	5	6	7	8
GEN. 1	Correct	0	309	43	0	0	199	0	0
	Total	931	2569	1364	1831	825	2481	1280	520
GEN. 2	Correct	109	300*	528	52	469	924		
	Total	235	1000*	1040	258	830	1104		
GEN. 3	Correct	306	88	59	195	34			
	Total	1284	1628	472	1240	1240			
GEN. 4	Correct	109	0	302	1428	125	67	75	
	Total	109	203	1039	1731	242	1052	528	

*Eggs were unable to be counted this day. These are estimates only.

II. (b) *Correct and total numbers of eggs laid in the amyl alcohol strain during the selection experiment*

	Days	1	2	3	4	5	6	7	8
GEN. 1	Correct	0	56	570	836	646	211	677	58
	Total	808	2300	1374	2692	852	2414	971	698
GEN. 2	Correct	152	1000*	0	0	0	192		
	Total	323	1300*	1659	1506	760	590		
GEN. 3	Correct	254	610	87	205	208			
	Total	1787	2227	1723	2917	1820			
GEN. 4	Correct	398	177	294	0	35	182	387	
	Total	997	1097	1238	1092	736	1106	1255	

*Eggs were unable to be counted this day. These are estimates only.

III. *Average migration % per generation*

Generation	% Migration	
	amyl alcohol strain	ammonium carbonate strain
1	4.5	4.8
2	6.6	9.3
3	6.4	6.1
4	6.6	9.0

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