Artificial Selection for Genetic Adaptation to Temperature Extremes in *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae)

Ernest B. White, Paul DeBach, and Morris J. Garber

Artificial selection was applied to several lines of the hymenopterous parasite *Aphytis lingnanensis* Compere (Aphelinidae) for more than 100 generations in an attempt to enhance tolerance to climatic extremes. All lines were drawn from an artificially enriched foundation culture. Culture III was subjected to low-temperature selection in each generation; Culture IV to high-temperature selection in each generation; Culture V to low- and high-temperature selection in alternate generations; and Culture VI to both high- and low-temperature selection in each generation. Four additional lines received similar temperature selection but were exposed to X-ray dosages. Selection in each of the nonirradiated lines resulted in improved tolerance, but irradiation did not measurably enhance tolerance to higher or lower temperatures.

When selection was terminated, Culture III required more than 64 hours exposure to 35°F to produce the LD_{50} which was reached by the control in 31 hours. Tolerance to high temperature was improved coincidentally.

High-temperature selection in Culture IV-"48" (successor to Culture IV) produced tolerance such that 33.1 hours exposure to 97°F was required to produce the LD_{50} which the control reached in 16.4 hours.

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ABSTRACT

Artificial selection was applied to several lines of *Aphytis lingnanensis* Compere (Aphelinidae) for more than 100 generations in an attempt to enhance tolerance to climatic extremes. Significant results included: (1) improved tolerance to temperature extremes as a result of selection; (2) irradiation failed to contribute measureably to enhanced tolerance; (3) the temperature tolerance was apparently well fixed genetically; and (4) general hardiness as indicated by adult longevity was improved coincidentally.

INTRODUCTION

CALIFORNIA RED SCALE, *Aonidiella aurantii* (Mask.) has been a long-standing major citrus pest throughout much of southern California. In 1947, the Department of Biological Control, University of California, Riverside, intensified its efforts to bring this insect under natural control. In November of that year, the world-wide search for new natural enemies produced a new species of *Aphytis* from China, known for several years as *Aphytis* "A" (DeBach, 1954), but later described as *Aphytis lingnanensis* Compere. It became established in some California locations as early as 1948 (DeBach *et al.*, 1950). Subsequently, it was laboratory reared for field colonization, culminating in the production of 26,000,000 adult females during 1954.

DeBach *et al.* (1955) demonstrated that *Aphytis lingnanensis* was an efficient parasite of California red scale on citrus in the coastal areas but not in the interior areas. Here, excessive mortality of the parasite in relation to the host, caused by low winter and high summer temperatures, prevented the parasite from maintaining economic control of the host. DeBach *et al.* (1955) established the limitations of climatic adaptation of *A. lingnanensis* and set the stage for the selective breeding program reported here.

In 1955, an effort began to select a strain or strains of *A. lingnanensis* more tolerant of low winter temperatures, high summer temperatures, or both.

Wilkes (1942) had probably been the first to artificially select a strain of parasites. He was able to select a strain of *Dahlbominus* (*Microplectron*) *fuscipennis* (Zett.), a parasite of the European spruce sawfly, *Diprion hercyniae* (Htg.), which was better adapted to cooler field locations. Other selective breeding programs with beneficial insects include the work of Simmonds (1947) who improved the sex ratio of laboratory cultures of *Aenoplex carpocapsae* (Cush.), a parasite of the codling moth, by breeding only those lines which produced more fe-

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males than the population mean; the work of Wilkes (1947) who selected for an improved biotic potential in his laboratory cultures of *D. fuscipennis* and succeeded in reducing the ratio of sterile males, improving the fecundity of females, improving longevity, and reducing variability in development, oviposition and length of adult life; the work of Urquijo (1951) who selected *Trichogramma minutum* Riley for improved fecundity and searching ability (ovotropism); the work of Wilkes *et al.* (1952) who selected a beneficial insect, *Macrocentrus ancylivorus* Roh. for DDT resistance and increased its resistance five- or sixfold after 18 generations; and the work of Allen (1954) who selected a strain of *Horogenes molestae,* a parasite of the oriental fruit moth, which became capable of reproducing on an unnatural insect host, the potato tuberworm. All these programs, as also does the work described in this paper, sought the solution to a practical problem.

DeBach (1958), discussing the possibilities of improving certain traits of beneficial insects by artificial selection, observed that a relatively low percentage of introduced species ever became successfully established and demonstrated from the literature that adaptive weaknesses occur in nature. He stated that "parasitic species which fail to become established or which become established but do not control the host successfully, nevertheless may be intrinsically highly efficient parasites." Such species failed to achieve economic control of the host, in some cases at least, because they adapted to a narrow range of environmental conditions, and could not adapt quickly to the new circumstances imposed upon them. De-

Bach pointed out that "the species with a high searching capacity but poor environmental adaptation offers the greatest promise for improvement through selection."

The selection program reported here followed the substantial efforts by this laboratory in the mass production and periodic colonization of *A. lingnanensis,* a parasite whose intrinsic capabilities had been carefully evaluated (DeBach, 1954) and whose climatic adaptations and limitations had been well established (DeBach *et al.,* 1955). It used the mass-production equipment, materials, and techniques for the propagation of the selected cultures and provided a widely colonized parasite to serve as an unselected control strain, referred to as Culture I.

Propagation of *A. lingnanensis* in the laboratory proceeded smoothly generation after generation at $80° ± 2°F$ and $50 ± 5$ per cent relative humidity. Reproduction in this species is biparental, with fertilized females (diploid) producing both sexes while unfertilized females produced haploid males only. Females outnumber males approximating two to one under normal insectary conditions where oleander scale, *Aspidiotus hederae* (Vallot), is provided as the host. Copulation normally occurs shortly after the female emerges and the single insemination provides adequate sperm for the entire oviposition period of the female. The life cycle of the parasite is about 14 days from egg to adult. A 48-hour oviposition period was normally used which resulted in adult emergence from the 13th through the 17th day with the peak occurring on the 15th day. Details on the biology and mass production of this species may be found in DeBach and White (1960).

METHODS

The success of any artificial selection program depends largely on the amount of genetic variability present in the or-

ganisms being selected. Variability in a given culture can be enhanced or maintained by several methods, principally

by (1) introducing into the culture genic material from as many geographic locations in the insect's range as is practicable; (2) maintaining and selecting large populations; and (3) subjecting the population to measured amounts of radiation thereby increasing the mutation rate and the probability of improving the organism's adaptability through selection. All three methods were employed to insure maximum variability in the foundation culture (designated Culture II) which was formed by combining four strains of *A. lingnanensis* from several geographical locations: (1) a mixture from Formosa; (2) the original Chinese strain (*Aphytis* "A"); (3) a strain from Texas; and (4) a mixture from Mexico. Care was taken to assure that the new mixed culture was composed of approximately equal proportions of the four geographical components and time enough was permitted for general mixing through at least four generations. From this foundation culture were drawn the four basic selection cultures plus four parallel cultures which were irradiated with an X-ray dosage.

Because climatic tolerance is probably polygenically inherited, it was essential for the success of the selection program to maintain high numbers and moderate levels of selection in the culture populations. Selection of polygenically controlled traits may be almost ineffective in small populations while in large populations the chances for successful adaptation of the desired type may be vastly improved. Therefore, we planned to maintain populations at levels that would subject at least 10,000 females to temperature selection in any generation. Actually, this number was usually larger but occasionally dropped below 10,000 in some cultures.

Parasites were selected and irradiated only as adults. This was necessary for practical considerations and also was compatible with the basic objectives of the selection program. In the

first place, only adult parasites could be bulk collected, and after treatment, an immediate definitive evaluation of the results as expressed in percentage of kill was available. Secondly, it was theorized that by improving the ability of adult parasites to survive climatic extremes and to reproduce after periods of inclement weather, the survival potential of the species should improve regardless of whether any new adaptive tolerance was expressed coincidentally by the immature stages. Finally, because a single copulation provided adequate sperm for egg fertilization throughout the entire ovipositional period of the adult female, the treatment of large portions of a population consisting of both sexes had the same effect as treating gravid females only. Therefore, males were not removed from the experimental population.

All cultures in the selection work were propagated in the mass-production cages described by DeBach and White (1960). These cages were inverted boxes with a top and two ends of wood, one side of glass (for light) and one of cloth (for ventilation). The open side was turned down over a white cardboard base. Oviposition in any generation was begun by placing into the cage the desired number of banana squash (*Cucurbita maxima*) infested by the oleander scale, *Aspidotus hederae* (Vallot), providing honey as a food source for the adult parasites, and adding the necessary adult parasites. Forty-eight hours later, oviposition was terminated by admitting a mixture of CO_2 and ether to the propagation cage. The anesthetized adults were removed on the white cardboard base and transferred either to a holding unit until sex ratio counts were completed or to a second propagation unit where a second 48-hour oviposition period was permitted. The double oviposition period was sometimes necessary to maintain the high level of reproduction essential to the aims of the selection program. In

any event, the sex ratio from adults that survived, as well as from those that succumbed to the test exposures, was always determined.

The filial adult progeny from both oviposition periods was made to emerge at the same time by cooling the population from the first oviposition period, so as to retard emergence for about two days. To accomplish this, the banana squash carrying this population was placed in a 45°F walk-in refrigerator for 18 hours on each of two consecutive days. The cooling treatment caused no measurable mortality (and, presumably, no further selection) if carefully timed to occur during the pupal stage of the immature parasites. Progeny were anesthetized and collected in the manner described before. Numbers of progeny were estimated from volumetric measurements of the collected adults.

When scheduled for selection, freshly collected adults were measured volumetrically and dispensed into 6 inch by 8 inch animal jars in groups of 12,500 individuals or less. The jars were streaked with honey to provide adequate food during treatment, and covered with slip cloth held in place by two large rubber bands.

Selection treatment of adults was designed to provide 50 per cent mortality in each generation of each selected culture, or 33⅓ per cent in each generation of each irradiated-selected culture which received irradiation. Because irradiation was to produce 25 per cent mortality among the immature progeny, reduction of the adult population by one-third reduced the size of the next generation to 50 per cent. Mortality at this level was the highest which could be utilized and still permit the maintenance of all selected cultures at the desired levels.

Selection was accomplished in cabinets in which the temperature was thermostatically controlled, but in which humidity was permitted to vary in response to temperature. Two methods were used to achieve the desired temperature in the cabinets: (1) By adding heat only, temperatures within a cabinet could be maintained at any desired level above room temperature, and (2) by cooling with a refrigeration unit, or heating with a radiant element, temperatures either below or above room temperature could be maintained. In practice, the first method gave better temperature control and was achieved even at 40°F by using a cabinet within a room maintained at 35°F.

Freshly collected adult parasites which were scheduled for irradiation were dispensed into plastic containers approximately 3 cm in diameter and about 1 cm deep, and covered with nylon cloth secured with a rubber band. The containers were sent to the California Institute of Technology, Pasadena, where they were irradiated under the supervision of Edward B. Lewis. Using a target distance of 15 cm and a 1 mm aluminum filter, the machine produced a 465 roentgen exposure per minute with plate voltage of 120 kv and plate current of 8 ma.

The effects of irradiation on *A. lingnanensis* had been evaluated by the authors in earlier experiments (DeBach and White, 1962). Therefore, exposure to the X-irradiation was timed so that the early generations in most irradiated cultures received a dosage of 750 r. units; however, because of X-ray induced variability and excessive mortality in some generations of cultures receiving repeated irradiation exposures, the dosage was reduced to 550 r. units after a few generations. This treatment level was considered adequate to produce significant genetic effects while not preventing more than one-fourth of the progeny of the X-irradiated generations of such cultures from maturing to adulthood.

Following treatment—simple selection or a combination of irradiation and selection—the surviving parasites were

separated from the dead and placed on fresh host material for the propagation of the ensuing generation. Initial determinations of the numbers involved in any given temperature treatment together with terminal estimates of survivors provided the gross mortality data discussed later. The mortality (expressed as percentage of those treated) resulting from successive selections provided the first estimate of acquired climate adaptation.

The following discussion of the establishment and experimental treatment of the selected cultures represents the planned program and, because certain variations from this plan were unavoidable, is a somewhat stylized outline of the work attempted. The first of the selection cultures, Culture III, was established as a low-temperature selection culture by subjecting 25,000 parasites from foundation Culture II to 43°F, for a period of 40 hours. Preliminary trials had indicated that these conditions could be expected to produce the desired level of mortality (50 per cent) in previously unselected adults. In each subsequent generation, the emerging progeny were subjected to similar low-temperature conditions for a similar or longer period of time.

In like manner, each of the other selection cultures was established by removing a large group of parasites from the foundation culture and subjecting them to the selection and irradiation conditions planned for that culture. Culture IV was subjected to a high temperature (90°F in the earlier generations; 94°F in later stages of the selection program) in each generation. Culture V was subjected to the low-temperature treatment in the first (parental) generation and to the high-temperature treatment in the second (first filial) generation, with alternating treatments administered to the ensuing generations. Both low- and high-temperature selection (each at a substantially reduced selection pressure) was

applied in each generation of Culture VI. Cultures III, IV, V, and VI formed the four basic selected cultures.

In addition, four parallel cultures were also drawn from Culture II, each of which received a low dosage of X-ray irradiation in every third generation. Culture VII received low-temperature selection (as did Culture III) but was also irradiated. Culture VIII was subjected to high-temperature selection (as was Culture IV) and to irradiation. Culture IX received the alternating treatments described for Culture V while Culture X received the combined treatments described for Culture VI but both IX and X were irradiated as well. Treatment details are discussed in a later section.

In addition to the mortality data resulting directly from parasite selection treatments, other evaluation methods were used. One of these provided the mortality curve and the LD_{50} for a given culture at a given point in time at one extreme temperature. To obtain the mortality curve, a series of samples was drawn from a given culture and subjected to extreme temperatures, not necessarily those used in selection. Periodic withdrawal from the treatment cabinet of subsamples yielded mortality data which permitted construction of the mortality curve for that culture at the given temperature. Logit analysis of these data provided an estimate of the LD_{50}, the standard deviation, and the slope. Curves described in the last six generations were combined into "terminal composite curves" providing a final or "terminal" estimate of time required at a given temperature to induce 50 per cent mortality among treated adult parasites. A t test of the differences between the $logD_{50}$ (the logarithm of the dosage which produced 50 per cent mortality) was conducted to demonstrate significant differences between the results from treated cultures and the control.

After termination of selection, the

longevity at 40°, 60°, and 80°F was measured by collecting freshly emerged adult parasites and dispensing them into five samples or replications. After honey had been provided for food, all five replicates were placed in the desired constant-temperature cabinet. During the course of the test and as long as any parasites survived, each sample was removed periodically from the environmental cabinet and held in the laboratory at 80°F, until the temperature within the test vials approached that in the laboratory. This stabilization period was found to be short for the samples held at 80°F, but reached one hour for samples held at 60°F, and 1½ hours for those held at 40°F. This stabilization period was essential to parasites held at the lower constant temperatures so they could resume near-normal insect activity including feeding. Cumulative mortality counts were made after equilibrium had been achieved, and all samples were checked during each of these observation periods to insure an adequate honey supply. The frequency of these periods was adjusted to the demands of the temperature response. Samples subjected to 40°F were removed to the laboratory, fed, and counted daily, those subjected to 80°F every other day, and those held at 60°F twice weekly.

The permanance of any acquired genetic adaptation to temperature extremes was evaluated as soon as established tolerance was evident. In the F_{81}, a portion of Culture VIII (irradiated and selected) was removed and, designated Culture VIII C, propagated continuously for 33 generations without further selection. Using the mortality curve technique, a check was made in each generation on temperature tolerance.

After selection and after the number of surviving cultures had been reduced to the four most successful lines, field evaluation was undertaken using standard techniques. Each culture was colonized in a comparatively isolated field population of California red scale on citrus which was under little or no apparent biotic pressure from other parasites. For each evaluation site three general types of controls were established, either singly or in combination. The DDT-check method (DeBach, 1946) was used in three of the four field trials. In two of these cases, a second check (an *Aphytis melinus* release and evaluation site) was used as a supplementary check measure. In the fourth field trial, the DDT-check was not used because the test sites had not enough trees. The control for this trial consisted of similar uncolonized sites.

RESULTS

Selection was applied to each parasite population generation after generation when permitted by culture circumstances. Selection treatment was omitted following a generation whose mortality, because of the treatment, was so high that the culture could not be maintained at the desired level. At one time or another, all cultures experienced such discontinuity in the planned selection program. Early in the selection work when irradiation and temperature selection were both applied

to a given population, the ensuing generation was frequently reduced to a level too low to permit selection in that generation. Eventually, omission of selection in the irradiated generations became the standard procedure.

Evaluation of the selection results was complicated by variations which apparently could be attributed only to the inherent variability in the species. Such variability demonstrated the effectiveness of the methods which had been employed to enhance variability. Other

workers have observed similar variability in cultures stemming from mixtures of geographically isolated acquisitions. Dobzhansky and Pavlovsky (1957) observed that "with geographically mixed populations the results [of propagation experiments] do not obey simple rules. The course of natural selection in such populations is often erratic." Despite the difficulties caused by these variations, the total changes induced by selection were large enough to demonstrate their significance. The figures 1 to 8 show graphically the running aver-

age of survival for cultures III through X as explained in the box below.

The selected *Aphytis* populations responded in nearly characteristic fashion to increases in selection pressure. A nonsubstantive increase caused no measurable response. A significant increase in selection pressure sometimes evoked little or no response in the first generation to which the increase was applied, but moderate to marked responses in the second and later generations. In the early phases of the selection program, the survival level declined in the first

How to Read Figures 1 to 8

Figures 1, 3, 5, and 7 show survival of Cultures III through VIA under the influence of selection at extreme temperatures. Figures 2, 4, 6, and 8 show survival of Cultures VII through X under the influence of both irradiation and selection at extreme temperatures. The graphs are paired so that each nonirradiated culture appears above its irradiated parallel line.

Average Survival. To minimize the generation-to-generation variability discussed above, the graphs show at each point (except the first two) the running average of data covering a five-generation span. The initial point in each graph represents the average adjusted survival in three generations, the parental generation (P) and the first two filial generations (F_1 and F_2). The second plotted point in each graph is the average adjusted survival in four generations (P, F_1, F_2, and F_3). The third point (F_2) and all points following represent the average adjusted survival in a five generation span. In some cases, where discontinuity in selection deprived us of data for some generations, the points represent the average adjusted survival for four, three, and even two generations. The number of generations actually represented in each point is keyed in each graph.

Time progression is shown along the horizontal base of each chart in terms of days since the start of the experiment, September 8, 1955. Corresponding dates are shown every 200 days.

Selection index, indicating survival in each generation, is given along the vertical ordinate. The use of an index became necessary in order to compensate for the periodic increases in selection intensity necessitated by the increase in tolerance to extreme temperature in each culture as selection progressed. In order to reduce the breeding population by 50 per cent in each generation, it became necessary to increase selection pressure from time to time as temperature tolerance improved. The selection index represents survival as adjusted for advancing selection intensity. It consists of two components, selection pressure and observed mortality. Selection pressure is measured in degree-hours (stress temperature times the hours of exposure). Stress temperature is the temperature above or below 65°F, the predetermined neutral temperature.

Example: The parental generation of Culture III was subjected to 43°F for 40 hours. Stress temperature was 22 (65 minus 43). Selection pressure (stress temperature, 22, times hours of exposure, 40) was therefore 880 degree-hours. The selection index (selection pressure, 880, times observed mortality, which in this case was .498) was found by multiplying these two figures (880 × .498), moving the decimal point two places to the left, and rounding, which gave a selection index for generation P as 4.38. Five-point averaging, as described above, reduced the figure to 3.94, the value graphed in figure 1.

Note: The transformation of data into an index and the average technique simplified the presentation of the observed results and smoothed much, but not all, of the variability in recorded observations. Because selection pressure was increased as selection progressed, this transformation in effect corrected the data summarized in figures 1 to 8 in such a manner that they show the approximate results that would have been achieved if all selection had been conducted at a standard selection pressure.
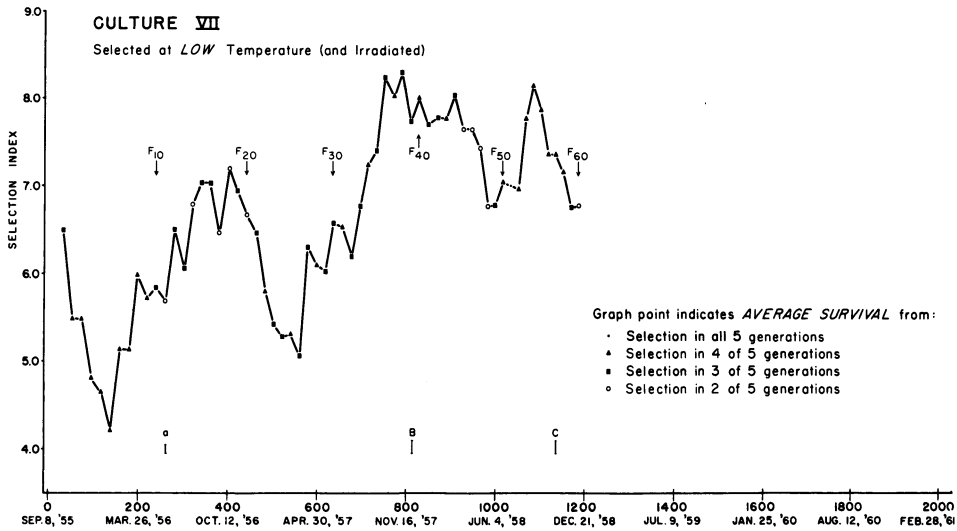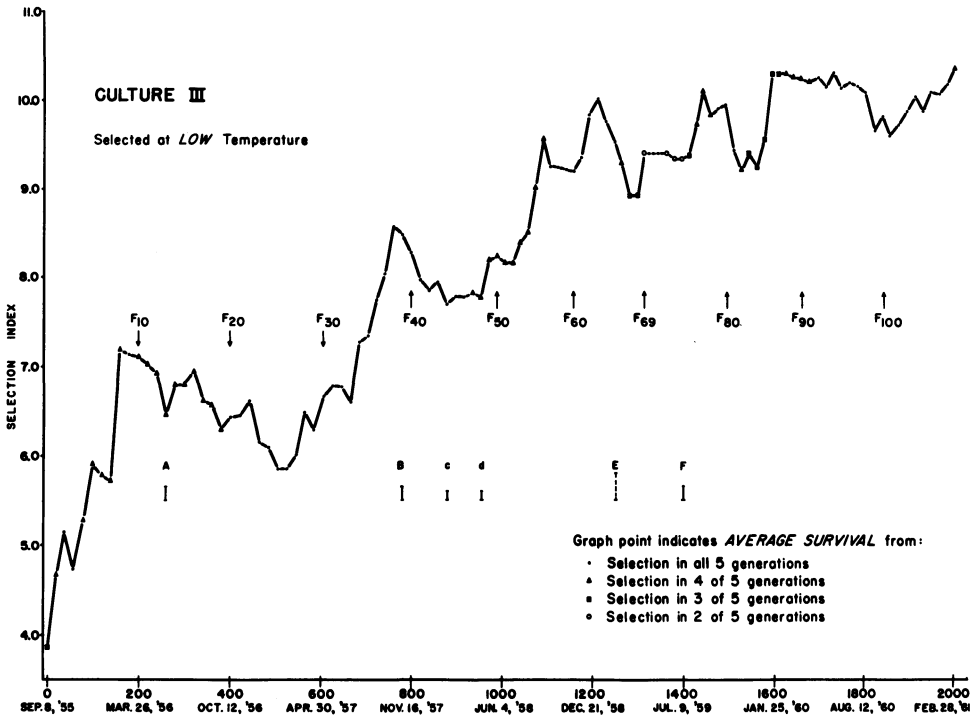
Figs. 1 and 2. Survival of cultures selected at low temperatures.
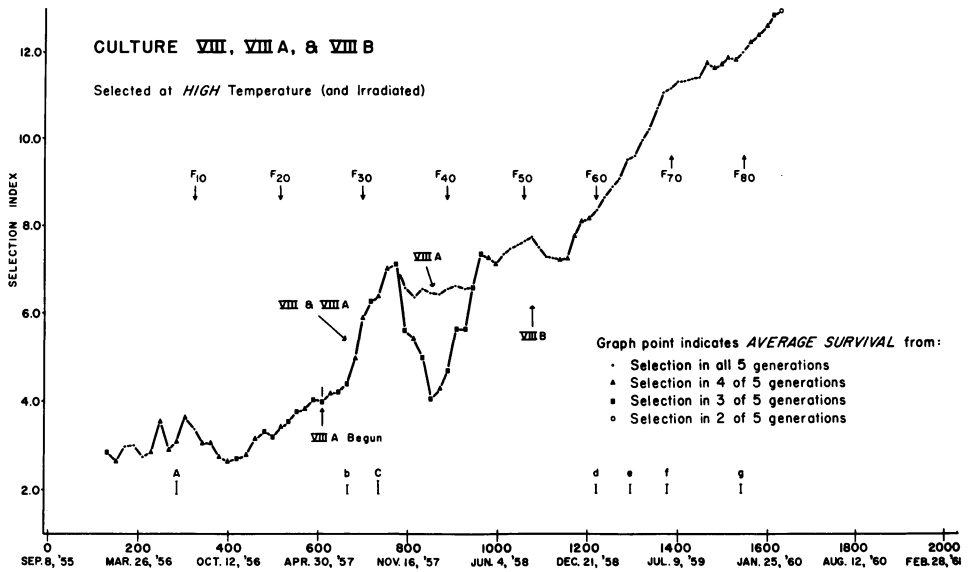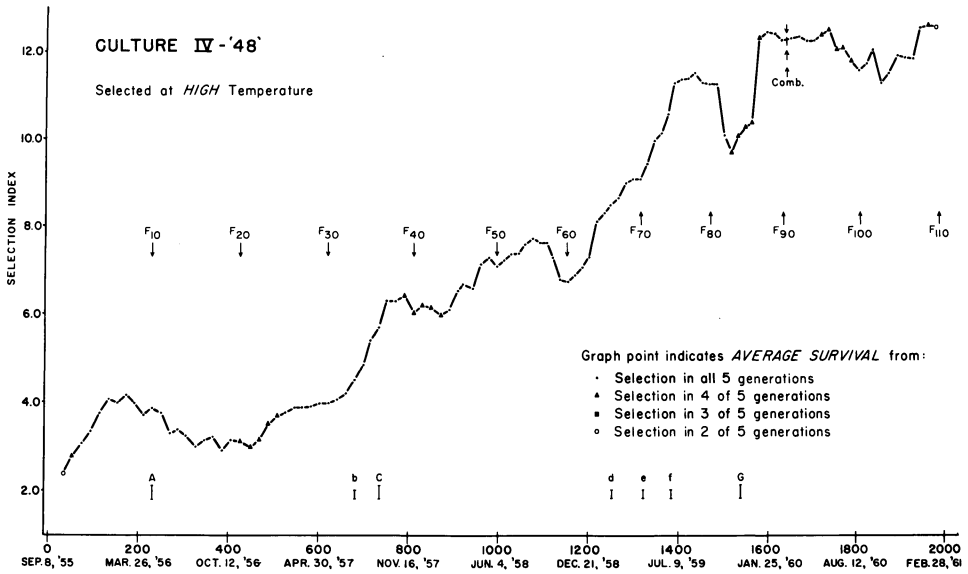
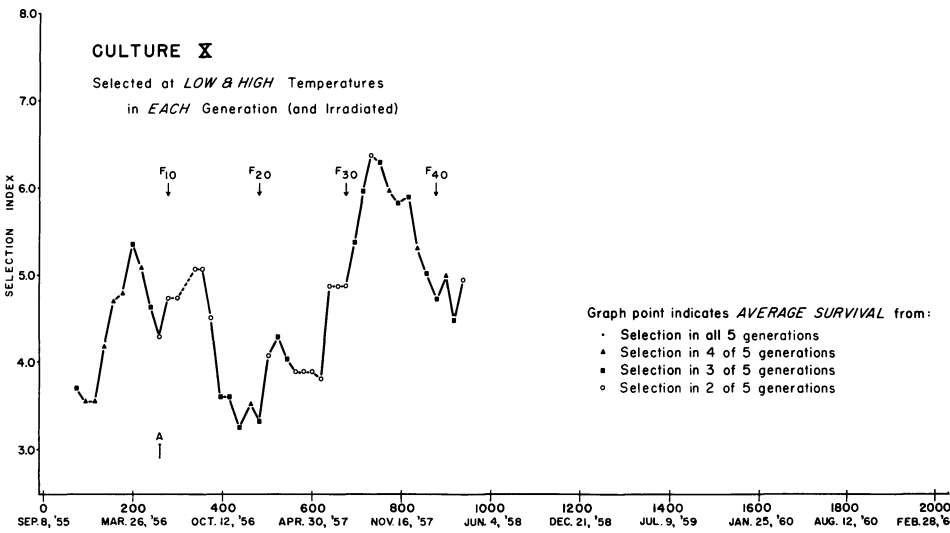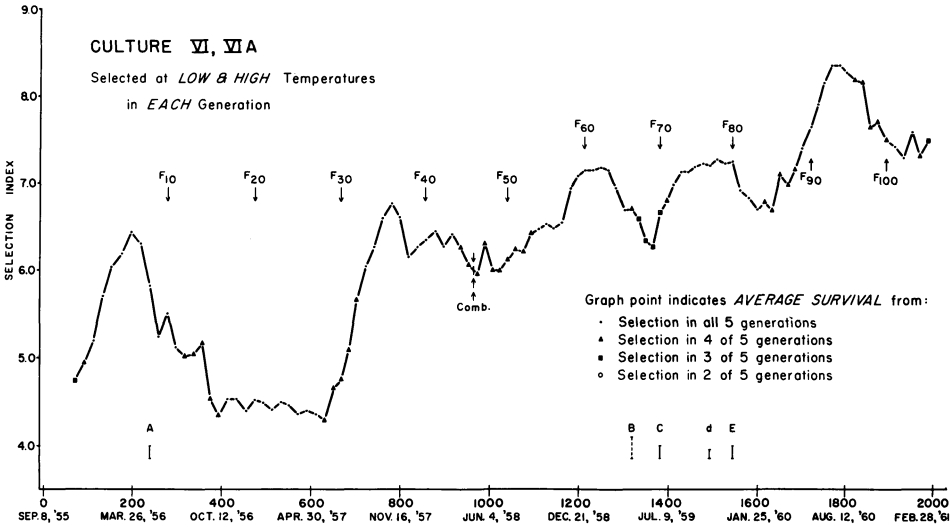Figs. 3 and 4. Survival of cultures selected at high temperatures.
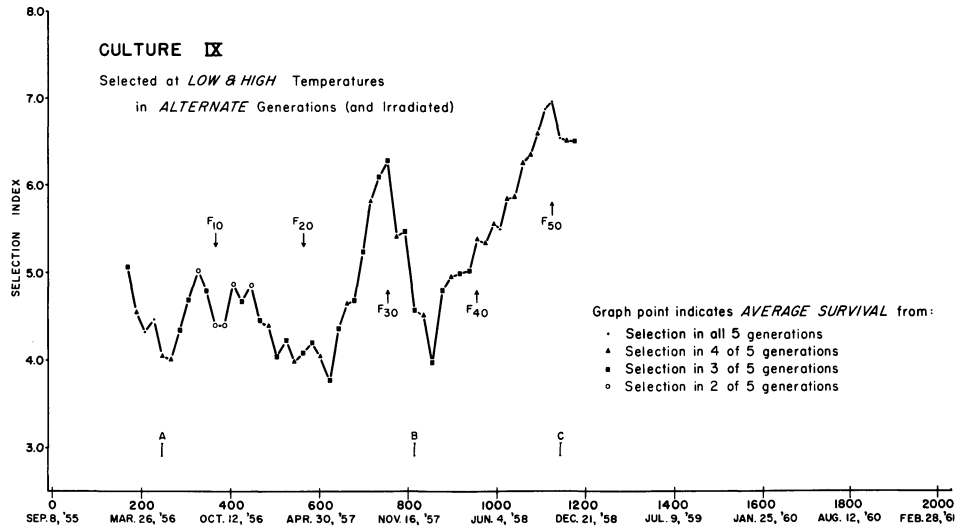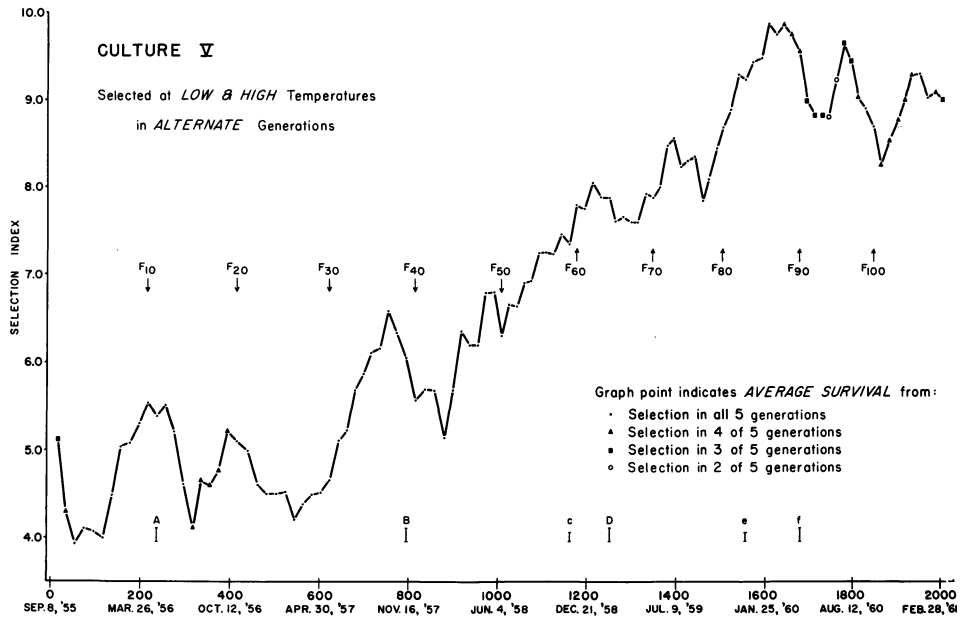
Figs. 5 and 6. Survival of cultures selected at low and high temperatures in alternate generations.

Figs. 7 and 8. Survival of cultures selected at low and high temperatures in each generation.

few generations in response to an increase in selection pressure, and then either continued downward or remained depressed for a number of generations. After prolonged selection, the marked response after an increase in selection pressure tended to subside more rapidly so that survival returned to or approached the normal level observed before the increase. Specific examples will be pointed out in the following sections dealing with the specific cultures.

The Nonirradiated Selection Cultures

Culture III. This culture, selected for tolerance to low temperatures, was treated at low temperatures in all generations when such treatment was possible. From September, 1955, when the culture was established, to March, 1961, when selection was terminated, 110 filial generations were completed, with selection applied in all but 13 generations.

Response to selection is plotted in figure 1. Note the remarkable increase in tolerance during the first ten generations. In the parental generation, selection treatment consisting of exposure to 43°F for 40 hours, resulted in 50 per cent female mortality. In the 11 generations which followed, survival improved rapidly in response to essentially the same selection pressure.

By reducing the treatment temperature to 40°F without changing the time of exposure, selection pressure was increased in the 13th generation, and was maintained at this level through the F_{38}. Because of the nature of the running average as used in the survival graph, the peak in the F_{10} and the decline in survival resulting from the increase in selection pressure, were reflected backward in the data as graphed for two generations. The vertical bar in figure 1 at A identifies the point at which this first substantive increase in selection pressure was made. The downward trend in survival resulting from this

increase in selection pressure continued through the 26th generation and probably represents a long period of reassortment and reorganization of genetic material.

After reversal of the downtrend, survival improved rapidly until the selection pressure was increased again in the F_{39} (B in figure 1). The dip resulting from this increase was smaller and shorter than the first.

Two further increases in selection time were made, one in the F_{44} (c) when the use of a 46-hour selection time was initiated, and again in the F_{48} (d) when the time of exposure to 40°F was extended to 48 hours. Apparently neither of these changes substantially influenced this culture which had been selected for so many generations; for this reason, lower-case letters, c and d, are used. In the F_{48}, survival began to improve. Progress was erratic but rapid through the F_{65} when a change in selection conditions reversed the uptrend and almost terminated the strain.

Beginning with the F_{65} and extending through the F_{70}, an attempt was made to increase selection pressure by reducing the selection temperature to 35°F(E). Results of exposure to 35°F were only slightly evident in parasite mortality but were abundantly evident in the adverse sex ratio of the progeny of treated adults. Sterility induced in females so treated yielded progeny which were predominantly male, resulting in drastically reduced populations in the second generation following such treatment. Table 1 illustrates the effect of this low temperature exposure on the sex ratio. The first three generations are given to show the typical sex ratio experienced with the selected parasites of Culture III just before the reduction of the temperature to 35°F. The sex ratio in the F_{65} is well within the normal range because their parents had received the normal 40°F treatment. The sex ratio in the F_{66} was near normal in spite of the 35°F selection used in the

TABLE 1

THE EFFECT OF SELECTION AT 35°F
AND 40°F ON THE SEX RATIO OF
ADULT *APHYTIS LINGNANENSIS*
COMPERE (CULTURE III)

Generation	Sex ratio (females)	Selection conditions	
		Temperature	Time
	per cent	degree F	hours
F_{62}	84	40	48
F_{63}	79	40	48
F_{64}	85.5	40	48
F_{65}	74.5	35	35
F_{66}	70.5	35	35
F_{67}	41	35	$31\frac{1}{4}$
F_{68}	21	No selection	
F_{69}	70	No selection	
F_{70}	75	35	$31\frac{1}{4}$
F_{71}	22	No selection	
F_{72}	70.5	No selection	
F_{73}	78	No selection	
F_{74}	76	40	48
F_{75}	52	40	48
F_{76}	55.5	No selection	
F_{77}	82	40	48
F_{78}	69	40	48
F_{79}	78	40	48
F_{80}	77	40	48

F_{65}; this fertility level can only be attributed to the beneficial effect of accumulated low-temperature tolerance factors. However, after two consecutive generations of 35°F selection, the sex ratio declined sharply in the F_{67}, a fact which caused a reduction in the time of exposure to 35°F in that generation. Again, the sex ratio declined in the F_{68} causing an enforced suspension of selection for two generations, and this relaxation of selection permitted an immediate rebound to near normalcy of the sex ratio in the F_{69} and F_{70}. However, subjection of the F_{70} to the 35°F selection temperature, even for the reduced exposure time, caused the sex ratio to decline sharply in the F_{71} and suspension of selection became necessary for three generations. In the F_{72}, as a result of relaxation of selection, the sex ratio recovered and remained normal through the F_{74}. At this point, the normal 40°F selection was resumed (F in figure 1) and, somewhat surprisingly,

this milder temperature also caused a reduction in sex ratio which is apparent in the F_{75} and F_{76}. Suspension of selection in the F_{76} apparently permitted the culture to recover completely so that no further propagational difficulty was experienced. However, figure 1 shows the erratic recovery period which followed the F_{76} for approximately 10 generations. This reassortment period was apparently followed by a plateau beginning in the F_{88} and continuing until the termination of selection in F_{110}.

The general form of the survival graph for Culture III suggests three distinct slopes of selection, each slope constituting a major period during which the rate of increase of adaptation was relatively constant. The first (P through F_{10}) is extremely steep; the second (F_{26} through F_{38}) is less steep; and the third (from about F_{45} through F_{88}) is gradual in comparison with the first two.

In any case, the acquisition of substantial tolerance to low-temperature (40°F) exposure over the long selection period is apparent in the graph. The average survival index during the last 10 generations was 10.06 (based on the adjusted running averages used in the graph) and 10.72 in the final selected generation. These correspond to survival levels of 83.8 and 89.3 per cent, respectively, among females exposed to 40°F for 48 hours. Time-temperature mortality curves for the unselected control culture indicate that female parasites from the unselected parental culture would have experienced an estimated 19 per cent survival had they been exposed to these conditions.

Another technique to measure temperature tolerance in the selected culture used the "mortality curve" test described earlier. Beginning in the F_{41}, a mortality curve was occasionally developed for certain generations of Culture III at a low temperature (35°F). A logit analysis of these data provided the LD_{50} in hours (as well as certain

TABLE 2

COMPARISON OF SURVIVAL BETWEEN SELECTED (CULTURE III) AND
UNSELECTED (CONTROL) ADULT *APHYTIS LINGNANENSIS* COMPERE
AT TERMINATION OF SELECTION AS MEASURED AT TWO
EXTREME TEMPERATURES (35°F AND 97°F)

Item	35°F		97°F	
	Culture III	Culture I	Culture III	Culture I
	Time in hours to LD₅₀			
Range..........................	50.3–69.5	25.2–37.0	15.6–25.4	13.3–19.4
Terminal composite mortality....	64.1**	31.0	20.7*	16.4

* Significantly different from control value.
** Difference is statistically highly significant.

other information) for the culture in each of the generations sampled. The need to define the effects of low-temperature selection on possible coincident increase or decrease in high-temperature tolerance led to initiation in the F_{65} of tests to describe mortality curves at 97°F.

The data provided by the mortality curves obtained during the last six generations of selection was combined into a composite curve and subjected to logit analysis. The results for Culture III are summarized in table 2. As can be seen in the terminal composite $LD_{50}s$ for Culture III and for the control (Culture I), high-temperature tolerance did not decrease but was actually improved, presumably because tolerance accumulated coincidentally (see pages 184–85). Tolerance of the selected culture to the low temperature extreme was dramatically improved as indicated by the fact that exposure to 35°F for 64.1 hours was required to produce the LD_{50} while this mortality level was reached in the control culture in only 31.0 hours. Retention of selected tolerance, i.e., adaptation, will be discussed in a later section of this report.

The survival graph of figure 1 indicates that Culture III had obtained a substantial low-temperature tolerance due to selection by the time the first mortality curve was obtained in the F_{41}.

In the F_{41}, the LD_{50} as measured in Culture III was 44 hours compared to 26 hours in the unselected control measured at approximately the same time. The median estimate for the 20 samples measured from the control culture was 28.5 hours. By the time selection was terminated, however, Culture III provided a 35°F LD_{50} of 67 hours.

Culture IV–"48". Culture IV was established to obtain a strain adapted to high temperatures typical of summer conditions in southern California. A parasite population drawn from the basic mixed culture was subjected to a moderately high temperature (90°F) for 17 hours. The survivors of this selection treatment were then used to propagate the F_1 generation. In like manner, the F_1 and each ensuing generation (as condition of the culture would permit) was subjected to high temperature selection until the culture had been maintained for 90 filial generations. In the F_{91}, it was combined with Culture VIIIB for reasons stated later. The combined culture, designated Culture "48", was propagated and selected another 20 generations—through the F_{110}. Selection at temperatures of 90°F or above was applied in 106 of the 111 generations propagated.

Survival among the parental females (selected at 90°F for 17 hours) was 48 per cent. Filial generations 1 through 8

were selected at temperatures ranging from 90° to 92°F for periods from 17 to 20 hours; their survival ranged from 35 to 94 per cent.

In the F_9, the temperature was increased to 94°F, and by the F_{11} time of treatment was standardized at 18 hours (A, figure 3). These selection conditions remained without interruption through the F_{32}. Figure 3 shows the decline in survival induced by the higher selection temperature (94°) and a broad valley in the survival curve extending from about F_{10} to about F_{26} and also a substantial recovery in rate of survival by the time the F_{32} was reached.

Selection time was increased to 20 hours (at 94°F) in the F_{33} and to 30 hours in the F_{36} (b and C, respectively). These combined increases yielded only mild selection results as indicated by the shallow valley between F_{37} and F_{48}. Selection pressure was not further increased until the F_{66} when exposure time was raised to 35 hours (d). Exposure was increased to 40 hours in the F_{70} (e), to 44 hours in the F_{74} (f) and, finally, to 48 hours in the F_{84} (G). As indicated in the graph, of these later increases only the last had any measureable affect, either singly or *in toto* upon the rate of selection in a culture which had been selected for so many generations.

Culture IV was combined with Culture VIIIB (successor to Culture VIII, the irradiated, high-temperature selection culture), into Culture "48" when the impending cessation of selection prompted certain culture consolidations and terminations. Cultures showing poor responses to selection were terminated and selection was continued in only the most promising ones. Cultures IV and VIII showed approximately equivalent tolerances to extreme temperatures and so were combined. Progress of this combination is shown in figure 3, beginning at F_{91} and continuing until selection (at 94° for 48 hours) was terminated in the F_{110}. (Cultures VIII and VIII B will be discussed further in a later section.)

The general shape of the survival graph of Culture IV suggests two distinct slopes of selection, with the second terminating in a plateau. The first (P through F_8) is short and steep. The second, beginning at about F_{20}, continues erratically through some 65 generations, ending at about F_{85}. After combining the two cultures in the F_{91}, a period of rearrangement of genetic material in the mixed populations apparently resulted in an unstable plateau which persisted until selection was terminated.

The success of the selection process in Culture IV is evident in figure 3. Female survival in the first three generations (P, F_1 and F_2) averaged 45 per cent when exposure to a temperature of 90°F averaged 18 hours. From F_{83} until the end, selection consisted of exposure to 94°F for 48 hours. Under these much more extreme selection contions, and through this prolonged period encompassing 28 generations, survival averaged approximately 89 per cent. Control data indicate that probably no more than 26 per cent of the parental females could have survived the severe selection conditions used during the latter stages of the work.

The data yielded by the mortality curve tests conducted on Culture IV-"48" provided a periodic comparison of tolerance in the selected culture with that in the control. Most of the high-temperature (97°F) mortality curves were obtained on this culture after F_{65} and, by this time, adaptation to high-temperature conditions was well advanced. Terminal composite mortality curves were compiled just as in Culture III, and substantiated the significant improvement in high-temperature adaptation of this culture as the result of prolonged selection. The magnitude of this change is expressed in the terminal composite LD_{50} data shown in table 3. While the control (Culture I) required

TABLE 3
COMPARISON OF SURVIVAL BETWEEN SELECTED (CULTURE IV-"48") AND
UNSELECTED (CONTROL) ADULT *APHYTIS LINGNANENSIS* COMPERE
AT TERMINATION OF SELECTION AS MEASURED AT TWO
EXTREME TEMPERATURES (35°F AND 97°F)

Item	35°F		97°F	
	Culture IV-"48"	Culture I	Culture IV-"48"	Culture I
	Time in hours to LD₅₀			
Range.......................	30.4–34.0	25.2–37.0	28.5–43.3	13.3–19.4
Terminal composite mortality....	31.7	31.0	33.1*	16.4

*Difference is statistically significant.

only 16.4 hours to reach the LD_{50} at
97°F, the selected adults (Culture IV-
"48") required 33.1 hours of exposure
to reach the same mortality level. At
the other extreme, tolerance to low
temperatures was not affected.

Culture V. This culture was estab-
lished to broaden the temperature spec-
trum through which a selected strain
might live and reproduce. It received
selection at both low and high tempera-
tures applied alternately to successive
generations. Unlike the simple direc-
tional selection applied to Cultures III
and IV-"48", the alternating of stress
temperatures in this culture corre-
sponds to the "cyclic" selection de-
scribed by Thoday (1956) and is akin
to the "disruptive" selection of Mather
(1953).

Selection treatment, either hot or
cold, was applied to 103 of the 109
filial generations propagated. Survival
of treated parasites was unusually high
in the parental and first two filial gen-
erations. The selection dosage adminis-
tered in each case was designed to pro-
vide approximately 50 per cent survival
among treated females, but when this
parental generation was subjected to
43°F for 40 hours, 65 per cent of the fe-
males survived. The progeny of these
survivors, the F_1 population, was held
for 17 hours at 90°F, high temperature
treatment which again permitted an
extremely high female survival of 87

per cent. The F_2 was subjected to the
same low-temperature conditions used
in the parental generation resulting in
81 per cent survival. Exposure to 90°
for one additional hour (18 instead of
17) in the F_3 permitted only 30 per
cent survival, and terminated the series
of unusually high survival results. Be-
cause the first point on the culture his-
tory graph (figure 5) is the average
survival for the first three generations
(P, F_1 and F_2), the unusually high sur-
vival in this culture during those early
generations gave a remarkably high
first (parental)-generation data point.
The low survival in the F_3 generation
contributed modestly to the reduction
in the averaged survival level for the
F_1 generation data point, but to a
greater extent in the F_2 and F_3 data
points. After the F_3, selection condi-
tions were made increasingly severe
until 18 hours at 94°F was reached in
the F_{11} (A in figure 5) and 40 hours at
40°F in the F_{12}. Thereafter, these were
used as standard selection conditions
until the time of exposure to 94°F was
increased to 21 hours in the F_{39}.

After the decline in survival from the
early series of highs, survival as aver-
aged in figure 5 remained low through
the F_5 and then began to increase even
though slight increases in selection
pressure were being made concurrently.
The cumulative effect of these increases
in selection pressure became apparent

in the F_9 and continued to be apparent well beyond the F_{25}.

Bar B in figure 5 represents a three-facet increase in selection pressure. The increase of exposure time from 18 to 21 hours at 94°F in the F_{39} caused only slight response, so the F_{41} was subjected to 24 hours at 94°F. This increase coupled with an increase in low-temperature selection pressure in the F_{42} (accomplished by extending exposure time from 40 to 44 hours at 40°F) reduced survival significantly as evidenced by the sharp decline, beginning at F_{38} and continuing through F_{43}. Recovery which began in the F_{44}, was completed in less than 10 generations under constant selection pressure at the new level. A further increase of exposure time from 24 to 30 hours at 94°F (c) failed to produce any response. A mild response was registered when the exposure time at 40°F was increased from 44 hours to its final value of 48 hours in the F_{64} (D), but two further increases in high temperature selection pressure were made without substantive results: in the F_{83} (e), exposure time to 94°F was increased to 35 hours and, in the F_{89}, to 40 hours (f). The decline which followed was due to mechanical difficulties with the 40°F constant-temperature cabinet. In the F_{90}, a faulty thermostat triggered a series of circumstances which caused omission of selection in that generation and in four others of the last 18. These omissions contributed to the variability shown in those late generations and in the reduced number of data points averaged in the graph as plotted. These difficulties had been essentially rectified by F_{105}, five generations before selection was terminated.

Culture improvement as a result of prolonged selection is obvious in figure 5, but less dramatic than in Cultures III and IV-"48", mostly because of the high level of survival in the first three generations. Even though improvement appears to be only modest, survival in this culture was substantially improved at both temperature extremes, as can be illustrated by comparing survival in the final generations with that of the control culture. Actual female survival in the last three generations subjected to low-temperature selection (48 hours at 40°F) was 73, 80, and 67 per cent (average 74 per cent), and control data indicate that no more than 18 per cent of the original unselected parents could have survived this treatment. At the other temperature extreme, female survival in the last three generations subjected to high-temperature selection (40 hours at 94°F) was 92, 92, and 93 per cent (average 92.4 per cent) while control data indicate that probably no more than 33 per cent of the parental females could have survived selection treatment of comparable severity.

Results of the mortality curve tests substantiate these observations. The composite data collected at the two temperature extremes during the last six generations of selection are presented in table 4 and permit evaluation of the extent of improvement occurring in each direction of the temperature spectrum. Specifically, approximately 40 per cent more exposure time to 35°F was required to produce the LD_{50} in the selected culture than was required to produce the same mortality level in the control, and at 97°F, almost twice as much exposure was required to produce the LD_{50} in the selected culture as required with the control. Obviously, selection in Culture V resulted in a greater degree of tolerance to high temperature than to low; however, substantive improvement is demonstrated at both extremes.

Culture VI–VIA. Just as Culture V was established to broaden the temperature spectrum through which a parasite population could survive by selection at *first one and then the other temperature extreme in alternate generations* ("cyclic"), Culture VI was started with a similar objective but with a different

TABLE 4
COMPARISON OF SURVIVAL BETWEEN SELECTED (CULTURE V) AND
UNSELECTED (CONTROL) ADULT *APHYTIS LINGNANENSIS* COMPERE
AT TERMINATION OF SELECTION AS MEASURED AT TWO
EXTREME TEMPERATURES (35°F AND 97°F)

Item	35°F		97°F	
	Culture V	Culture I	Culture V	Culture I
	Time in hours to LD₅₀			
Range...........................	33.9–57.1	25.2–37.0	20–35.3	13.3–19.4
Terminal composite mortality....	43.0*	31.0	31.7*	16.4

* Difference is statistically significant.

experimental approach. Selection treatment, in this culture, was applied at *both extremes in each generation*. The treated population in each generation was subjected to low temperature and then to high temperature, remaining in each for carefully predetermined periods, so that mortality produced by the second treatment was simply added to that produced by the first, and surviving insects were survivors of both treatments. This does not correspond to the disruptive selection of Mather (1953) because, by definition, disruptive selection is operating when several optima are favored (Mather, 1955) and usually requires selection of more than one class of individuals (Thoday, 1959). However, it appears to be closely related to this type of selection.

The combined selection treatment was applied to every generation in which culture condition would permit, and as a result, 97 of the 105 filial generations propagated received selection. After propagation through 45 filial generations, this culture was combined with Culture X (the irradiated parallel culture) redesignated as Culture VIA.

In practice, low-temperature selection was applied to a given population and was followed immediately by exposure to high-temperature conditions. Time of exposure to each extreme was adjusted before initiation of the selected culture to provide mortality of

about 25 per cent of the total number of females exposed; in this way, total mortality among females approximated 50 per cent. Actually, 58 per cent of the parental females survived selection treatment consisting of 26 hours at 43°F followed by exposure to 90°F for 5 hours. Survival increased dramatically during the first few filial generations even though selection pressure was increased gradually. By the time F_8 had been reached, selection conditions had been standardized, so that treatment in this and many subsequent generations consisted of exposure to 40°F for 28 hours and to 94°F for 4 hours. This set of selection conditions reduced survival to the desired level, where it remained through the F_{30}. Recovery to higher levels of survival at F_{36} together with an unexplained modest decline (F_{37-43}) followed.

In the F_{46}, Culture X (discussed later) was combined with this culture to form Culture VIA. Variability in response to treatment increased temporarily as a result, but then a prolonged period of improving survival followed (F_{50-62}).

An attempt to increase selection pressure by reducing the low temperature from 40° to 35°F in the F_{66} and F_{67} created the same problems in this culture that were experienced when Culture III was subjected to similar selection temperatures. Survival was af-

COMPARISON OF SURVIVAL BETWEEN SELECTED (CULTURE VI-VIA) AND
UNSELECTED (CONTROL) ADULT *APHYTIS LINGNANENSIS* COMPERE
AT TERMINATION OF SELECTION AS MEASURED AT TWO
EXTREME TEMPERATURES (35°F AND 97°F)

Item	35°F		97°F	
	Culture VI-VIA	Culture I	Culture VI-VIA	Culture I
	Time in hours to LD₅₀			
Range...........................	46.3–64.3	25.2–37.0	20.8–23.8	13.3–19.4
Terminal composite mortality....	57.4*	31.0	22.5*	16.4

*Difference is statistically significant.

fected only moderately but the sex ratio
of the culture was drastically reduced
so it became necessary to propagate two
generations without selection to bring
the female population back to the nor-
mal working level.

When selection was resumed in the
F_{70}, treatment, conditions were the
same as those to which the F_8 had been
subjected, namely, 28 hours at 40°F
plus 4 hours at 94°F. With survival
improving steadily, a modest increase
was made in the F_{77} (to 30 hours at
40°F and 4¼ hours at 94°F). Reaction
to these combined increases was only
moderate but no further increase was
attempted.

Overall culture improvement is evi-
dent in figure 7. Survival as averaged
for the final data point is almost twice
as high as that for the parental genera-
tion, although the final generation was
subjected to substantially more rigor-
ous selection conditions.

Further evaluation of culture im-
provement is provided in the data from
the mortality curve tests shown in table
5. At 35°F, the LD_{50} provided by the
composite mortality curve at termina-
tion of selection was 31 hours for the
control, while 57.4 hours were required
to achieve the same mortality level
among selected parasites. Improvement
at 97°F was not so dramatic—16.4
hours for control parasites to reach the

LD_{50}, and 22.5 hours for selected (Cul-
ture VI–VIA) parasites.

Obviously, a degree of success was
achieved in both attempts to broaden
the temperature spectrum of tolerance
displayed by two insect populations.
However, because of the specific tech-
niques employed and the selection
treatment dosages used, high-tempera-
ture tolerance was improved to a
greater extent in Culture V than was
low-temperature tolerance. For the
same reasons, the converse was true in
Culture VI–VIA where low tempera-
ture tolerance was improved to a sub-
stantially greater degree than was tol-
erance to high temperatures (see dis-
cussion on page 185).

The Irradiated Cultures

Culture VII. The first irradiated cul-
ture was originated as a low-tempera-
ture selection culture to parallel Cul-
ture III. Low-temperature selection was
applied in each generation together
with irradiation treatment in the paren-
tal and each third filial generation inso-
far as culture condition would permit.
Because of X-ray-induced variability
and excessive mortality in some gen-
erations, it became necessary to omit
both selection and irradiation from
three of the first 12 generations and
eight of the 60 propagated.

The parental generation was sub-

jected to irradiation (750r) and to low-temperature selection consisting of exposure to 43°F for 32 hours. Survival was 86 per cent compared to the 66.7 per cent expected value. Normal low-temperature treatment (40 hours at 43°F) was applied in the next two filial generations during which survival remained high but, when the F_3 was subjected to irradiation and normal selection, survival dropped sharply to 27 per cent causing omission of selection in the F_4. Mechanical difficulty in the 40°F cabinet caused the F_5 to be subjected to approximately 35°F which resulted in low survival. Normal irridiation and selection were then applied through the F_8, but omission of treatment was necessary in the F_9. Normal selection was applied on the F_{10}.

Beginning with F_{11}, selection pressure was increased in the nonirradiated generations by reducing the treatment temperature to 40°F. The resultant mortality made it necessary to omit all treatment in the F_{12}. Then, beginning in the F_{13}, selection treatment was omitted in each irradiated generation.

Propagation continued without further change in irradiation or selection treatment through the F_{38}. Exposure time was increased in the F_{39} (B, figure 2) to 44 hours (from 40), a change which induced a substantial reaction as measured in the survival level of the next dozen generations. Survival was still depressed in response to this higher selection pressure when the culture received its 16th (and final) irradiation in the F_{46}. Two generations without selection were needed to recover from this irradiation treatment, after which survival began to improve. Selection pressure was intensified once again in the F_{57} (C) by increasing exposure time to 48 hours, and again culture survival in response to selection declined sharply so selection treatment had to be omitted in the F_{59}.

Comparisons of Culture VII with its nonirradiated parallel culture (III) led to termination of this irradiated culture in the F_{60}. Survival in response to low-temperature selection (48 hours at 40°) in the last three generations selected ($F_{57, 58}$, and $_{60}$) averaged 64 per cent while Culture III parasites averaged 77 per cent survival during their F_{57-60}. Unselected control parasites would have succumbed to such an extent that only 19 per cent would have been expected to survive. Although Culture VII had shown substantial improvement in tolerance to low temperature, its distinct inferiority to the nonirradiated parasites of Culture III prompted its termination. As noted earlier, survival in Culture III continued to improve so that terminal female survival was 89 per cent in response to standard low-temperature selection conditions.

Culture VIII, VIIIA and VIIIB. To establish an irradiated parallel to the high-temperature selection culture (IV), Culture VIII was begun. High-temperature selection was applied in each generation and irradiation in every third filial generation insofar as culture condition would permit. Selection or irradiation was applied to all but two of the 44 generations through which the culture was propagated before it was modified.

The parental parasite population was irradiated (550r) and subjected to selection treatment consisting of exposure to 91°F for the reduced time of 15 hours. Resulting female survival was 78 per cent, a level substantially exceeding the 66.7 per cent desired. Normal selection pressure levels in the first two filial generations provided approximately the desired survival level. Irradiation combined with lower-than-normal selection pressure applied to the F_3 provided 49 per cent survival, a value lower than the desired level but adequate to permit uninterrupted culture propagation and selection.

Normal selection conditions, applied to nonirradiated generations, was in-

creased in the F_8 (A, figure 4) by increasing the temperature from 91° to 94°F for the standard 18-hour exposure period, and beginning with the F_{14}, selection was omitted in the irradiated generations.

In the F_{25}, a substantial fraction of the culture was used to establish a sister culture designated Culture VIIIA. This culture receiving normal selection but no further irradiation, was established to conserve the tolerance which had accumulated as a result of 25 generations of selection if continued irradiation should prove detrimental in the original culture.

Propagation of both cultures was continued without further change in treatment until a modest and apparently ineffective increase in selection pressure was instituted in F_{28}. This change consisted of an increase in the time of exposure to 94°F from 18 to 20 hours. In F_{31} and F_{32} (C) successive increases in exposure time to 24 and then 30 hours became highly effective in Culture VIII (which was still receiving periodic irradiation) by the F_{37} and F_{38} as demonstrated in the sharp decline of survival in the graphline of this culture in figure 4. Recovery in survival was essentially complete when, in the F_{44}, the original culture was recombined with Culture VIIIA, to form Culture VIIIB.

In the absence of any further irradiation treatment, Culture VIIIA continued to respond well as shown in figure 4 (at VIIIA). The selection pressure increases applied to Culture VIII in the F_{28}, F_{31} and F_{32} were also applied to Culture VIIIA but found less response, as indicated by reduction in survival level. Survival in the F_{43} was close in both cultures after receiving identical selection treatments, so they were recombined in F_{44}. Irradiation was discontinued at this point, so that the combined culture (VIIIB) continued to receive only high-temperature selection treatments applied in each generation in which culture condition would permit. Propagation continued through the F_{85} and selection was omitted in only three of the 41 generations reared after recombination.

During these last 41 generations, selection pressure was increased on four occasions. The time of exposure to 94°F was increased from 30 to 35 hours in the F_{59} (d), to 40 hours in the F_{64} (e), to 44 hours in the F_{69} (f) and to 48 hours in the F_{79} (g). These increases caused only minor responses in survival level. Survival continued to improve steadily so that in the last three selected generations, 92.7 per cent of the females exposed to 94°F for 48 hours survived. This compared with 26 per cent survival among control parasites receiving the same treatment.

At this point in time, Culture IV (the nonirradiated high-temperature culture) had completed 90 filial generations. Female survival in generations F_{89} and F_{90} of that culture, while undergoing identical selection conditions as those used in VIIIB, was 93 per cent. Therefore, the two cultures were combined to form culture "48", as discussed earlier.

Culture VIIIC. This culture was established to observe any changes which might occur in temperature tolerance after selection was terminated, Culture VIIIC was taken from a substantial fraction of Culture VIIIB in the F_{80}, maintained without further selection through 18 months and 33 generations, and terminated in the F_{113}. The fate of accumulated tolerance will be discussed in a later section.

Culture IX. The goal here was to provide a culture tolerant to a broad temperature spectrum, with selection at the temperature extremes applied in alternate generations (cyclic as in Culture V), but upon which the effects of X-ray irradiation could be imposed. Fifty of the 54 generations propagated received either selection or irradiation (and two of the 50, the parental and F_3, received both).

As the result of irradiation (550r) and selection treatment at the only slightly reduced exposure time of 36 hours to 43°F, survival in the parental generation was low (45 per cent when 66.7 per cent had been desired). However, in response to normal selection conditions in the F_1 (18 hours at 92°F) and F_2 (40 hours at 43°F) survival was excessively high (86 per cent in both cases). The F_3 received irradiation and a high-temperature treatment of reduced intensity which permitted survival to remain high. An increase in low-temperature selection pressure caused by decreasing treatment temperature from 43° to 40°F in the F_4 (A, figure 6) reduced survival to a low but acceptable level (43 per cent). Similarly, an increase in high-temperature selection pressure imposed upon the F_5 by increasing treatment temperature from 90° to 94°F for the normal 18 hours yielded nearly ideal survival of 52 per cent.

Selection in irradiated generations was first omitted in the F_{10}, but propagation continued without further changes in treatment until the high-temperature exposure was increased from 18 to 21 hours in the F_{33} (B). Response to this increase is shown in figure 6, but the effect is reflected back through F_{31}. Recovery from this survival decline was well advanced when irradiation was terminated with treatment of the F_{40}.

The final change in selection pressure was applied in the F_{51} (C) when high-temperature exposure was extended from 21 to 30 hours. Response to this increase is again reflected backward two generations, and survival was still declining when the culture was terminated after F_{53}.

The culture was discontinued after its accumulated tolerance was compared at the two temperature extremes with that of Culture V at that time. Female survival in the last two generations selected at high temperature (the F_{51} and $_{53}$) averaged 67 per cent, while in

Culture V 79 per cent of the females survived identical treatment in corresponding generations. Both these two survival figures were higher than the 61 per cent of unselected (control) females surviving the same selection treatment, illustrating the difference between the two cultures in tolerance to high temperature.

Low-temperature tolerance differences between the two cultures are not so directly comparable, because Culture IX was still being exposed to 40°F for 40 hours while Culture V was being (and for several preceding generations had been) exposed to 40°F for 44 hours. Survival level in both cultures was approximately the same. However, since Culture V was being subjected to the more rigorous selection conditions, the accumulation of low-temperature tolerance factors was obviously further advanced in Culture V than in Culture IX. Because the nonirradiated Culture V was superior at both temperature extremes, Culture IX was discontinued after propagation of 53 filial generations.

Culture X. Originating as an irradiated parallel to Culture VI, this culture was selected at *both* temperature extremes in each generation and, in addition, was irradiated in the parental and each third filial generation when culture conditions would permit. Propagation proceeded through 44 filial generations, eight of which received neither irradiation nor selection. Irradiation was applied in the parental and 12 of the filial generations.

The parental generation was exposed to X-irradiation and then subjected to selection at both temperature extremes. It was exposed to 43°F for 26 hours, then to 90°F for five hours. Survival of parental females was 56 per cent, lower than the desired 66.7 per cent level. However, in response to selection treatment only in the next three generations, survival ranged from 43 to 55 per cent,

which was acceptable since a 50 per cent survival level was desired.

High survival in the F_5 through F_7 led to an increase in selection pressure in the F_9 (A, figure 8), the new treatment consisting of a 28-hour exposure at 40°F and a four-hour exposure at 94°F in the nonirradiated generations. Beginning in the F_{10}, the practice of treating irradiated generations was discontinued and no further changes in treatment were made for the rest of the time this culture was propagated.

Response to the single substantial increase in selection pressure is shown in figure 8. Because of the averaging technique used in preparing the graph, the decline in survival is reflected back to F_8 and even to some extent in F_7. This decline was disrupted by a survival increase for five generations (F_{10-14}) which is difficult to explain on the basis of the data alone. Presumably, some cryptic combination of tolerance factors caused the unusually high survival in the F_{12} which contributed so heavily to the increase. In F_{15} the decline in survival was resumed and reached its lowest point in about the F_{20}. For several generations survival remained low, followed by a brief but spectacular period of increasing survival.

The decline of the last eight or ten generations propagated cannot be explained on the basis of any change in culture treatment. It could have been caused by some cryptic accumulation of irradiation-induced lethals.

Based on an occasional glimpse of promise in this culture, rather than on any demonstrable merit, the line was merged with Culture VI in the F_{45} to form VIA, a culture already discussed.

Failure of irradiation to contribute to selected tolerance. All four irradiated cultures (except VIIIC) were discontinued through merger with another culture or by termination before the selection project was concluded: Cultures VII and IX were discontinued;

Culture VIII was combined with Culture IV; and Culture X with Culture VI. None of these achieved a tolerance level high enough to indicate that any influence, other than simple selection for temperature tolerance was acting upon the culture. In fact, most of the irradiated cultures were maintained with some difficulty. The exception, Culture VIIIC, was maintained for many generations without selection or other treatment for the particular purpose of exploring the fate of selected tolerance after relaxation of selection treatment.

Substantially more variability was encountered in the X-ray treated cultures than in the nonirradiated ones; however, the vast majority of mutations and all chromosomal effects were apparently detrimental to population fitness and were eventually removed or screened out as a result of the selection process.

That irradiation did not contribute to tolerance to extreme temperatures was to be expected because most mutations have been considered deleterious. The literature abounds with examples, only two of which are mentioned here. Muller (1950) pointed out the accumulation of lethals in irradiated stocks and Clayton and Robertson (1955) concluded that utilizable radiation-induced variability is small. The entire subject has been reviewed by Grosch (1962). Because selection as applied in this work was to facilitate accumulation of polygenic temperature tolerance factors, only a formidable single gene mutation would have produced the recognizable survival differentials which had been desired.

Effects of Selection Demonstrated by Mortality Curve Data

Mortality curves were described from occasional samples drawn from the cultures undergoing selection. Results of
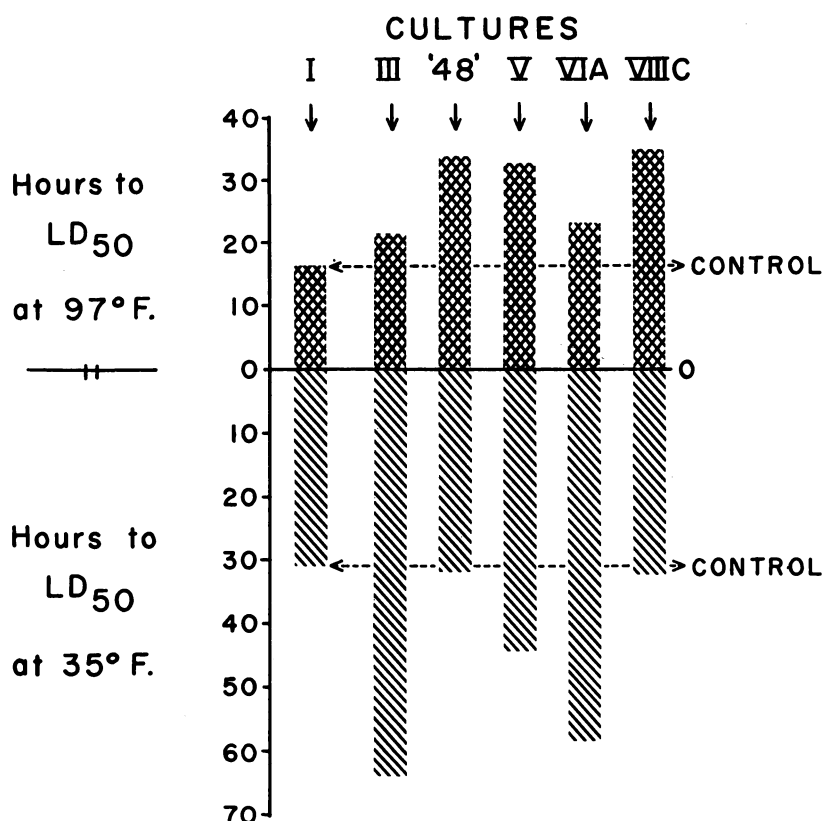
Fig. 9. Temperature tolerance profile derived from the terminal composite curves. The profile shows the number of hours required at the two extreme temperatures to produce the LD_{50} (50 per cent mortality) and indicates the relationship of this response to that of the control culture.

these tests are summarized in figure 9 and table 6.

The low-temperature results, as graphed in figure 9, reveal that Cultures IV–48 and VIIIC, both selected at high temperature, were unaffected with regard to tolerance at low temperature. Culture III underwent the greatest change in tolerance to low temperature reaching the LD_{50} in more than twice the time that was required for the control. Cultures V and VIA, selected for increased tolerance to both low and high temperatures, were also substantially more tolerant to the low-temperature test conditions than were control parasites but less so than Culture III.

In response to high temperatures, tolerance in all selected cultures was improved. Cultures IV–48 and VIIIC were essentially identical at the end of the selection program and both showed substantial improvement in tolerance to high temperature, each requiring more than twice the time at 97°F to produce the LD_{50} that was required by the control culture. Tolerance in Culture V was almost as good, but Culture VIA did not approach the level of tolerance achieved by these three.

The improved high-temperature tolerance displayed by Culture III was a surprising result of prolonged selection to low temperature. It was tempting to explain it simply as improved vigor but a better answer is suggested by the data in table 6. That table reveals that Cul-

RESULTS OF MORTALITY CURVE TESTS

Culture	LD_{50} at 35°F	Ratio[†]	LD_{50} at 97°F	Ratio[†]
	hours		*hours*	
I-Control...........................	31.0	1.00	16.4	1.00
III.................................	64.1[**]	2.07	20.7[*]	1.26
IV "48".............................	31.7	1.02	33.1[*]	2.02
V...................................	43.0[*]	1.39	31.7[*]	1.93
VI A................................	57.4[*]	1.85	22.5[*]	1.37
VIII C..............................	32.2	1.04	34.9[**]	2.13

[*] Difference is statistically significant.
[**] Difference is statistically highly significant.
[†] LD_{50} Result ÷ Control Result.

ture III required 20.7 hours or 1.26 times as long to produce 50 per cent mortality at 97°F as did the control after termination of the selection program. This substantial improvement suggests that the accumulation of low-temperature tolerance factors during the prolonged selection period modified the gene complement in the population in such a manner as to improve survival, at least in a modest way, when samples were subjected to high temperatures. In other words, selection at low temperature generation after generation resulted not only in improved low-temperature tolerance but in an accumulation of factors which, somehow, improved tolerance to high temperatures as well.

Figure 9 shows that Culture V was more heat tolerant but less cold tolerant than was Culture VIA. The technique employed in the selection process with Culture VI-VIA was probably inadvertently biased in the direction of low-temperature selection. It was uniformly the same in each treated generation: the sample population was placed first in the low-temperature cabinet for a predetermined period, then transferred to the high-temperature cabinet for another predetermined period, and each segment of the treatment was designed to kill one-third of the female population exposed to that treatment. Theoretically, this appears sound but the high-temperature treatment may

have acted first on those individuals already weakened by the previous low-temperature treatment (in effect, adding to the low-temperature mortality) before the high temperature even began to affect the healthy survivors of the low-temperature treatment. In other words, exposure to high temperature immediately after low-temperature treatment possibly enhanced the effect of the low-temperature selection first, and produced high-temperature mortality only secondarily. This compounding of low-temperature mortality would not have been detected by measurement techniques employed and, therefore, is suspect as a contributing factor to the final tolerance spectrum achieved by Culture VIA.

In summary, figure 9 shows that (1) selection for low-temperature tolerance in Culture III was eminently successful; (2) selection for high-temperature tolerance in Cultures IV–48 and VIIIC was also eminently successful; and (3) selection for broad-spectrum tolerance in Cultures V and VIA was highly successful, but the tolerance ranges of these two cultures were markedly different when selection was terminated.

Effect of Selection on Adult Parasite Longevity and Vigor

The longevity tests on adults from the surviving cultures were distinctly different from the "mortality curve"
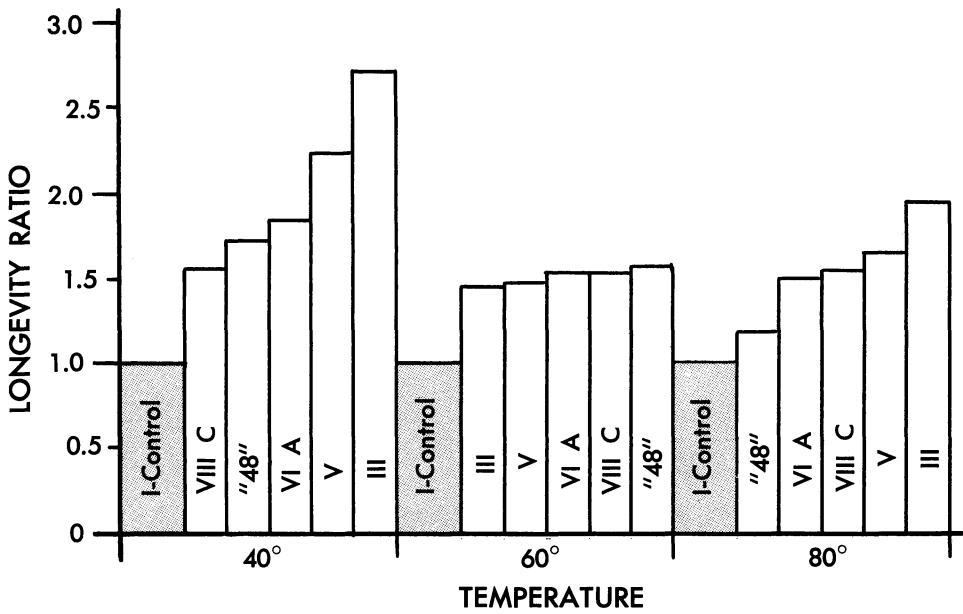
Fig. 10. Longevity profile, demonstrating one facet of "hardiness" by comparing longevity of each selected line (after selection was terminated) with that of control.

tests just described even through mortality (the LD_{50}) at a given temperature was used as a criterion in both. These longevity measurements were conducted to investigate population hardiness or vigor (those attributes other than tolerance to temperature extremes which permit the population to flourish). This series of tests was designed to measure longevity at three temperatures: one, 40°F, was low enough to permit temperature-induced mortality, but the other two, 60° and 80°F, were mild enough to avoid temperature-induced mortality, thus permitting unspecified hardiness factors to become fully effective and to be displayed in the longevity of samples under conditions presenting no temperature duress.

Results of these tests (figure 10) are expressed in terms of a "longevity ratio"—the ratio of survival between the selected parasites of a given culture at a given temperature and the control parasites under identical conditions. To assist in this evaluation, the survival figures (expressed as the LD_{50} in days) are given in table 7.

The consistency with which all selected cultures outlived the unselected control at all three temperatures checked was the amazing and unexpected aspect of these results. The obvious conclusion is that during the process of selection for tolerance to extreme temperatures, an accumulation of factors for general hardiness (or at least for longevity) occurred concurrently that made all cultures survive longer than the control parasites under conditions of the test.

Results at 60°F illustrate this general hardiness most clearly. The 60°F temperature approached the optimum for longevity in the species: at this temperature little or no mortality occurs as a direct result of temperature acting upon the parasites in the sample. In the absence of a mortality-inducing temperature, normal longevity factors become effective. The fact that all selected cultures lived substantially longer than the unselected control dem-

TABLE 7

RESULTS OF TESTS DESIGNED TO MEASURE THE LONGEVITY OF THE
SELECTED CULTURES AFTER TERMINATION OF SELECTION

40°F			60°F			80°F		
Culture	Longevity*	Ratio†	Culture	Longevity*	Ratio†	Culture	Longevity*	Ratio†
	days			*days*			*days*	
I-Control	1.8	1.0	I-Control	42.0	1.0	I-Control	9.7	1.0
VIII C	2.8	1.56	III	61.0	1.45	"48"	11.5	1.19
"48"	3.1	1.72	V	61.5	1.46	VI A	14.5	1.50
VI A	3.3	1.83	VI A	64.0	1.52	VIII C	15.0	1.55
V	4.0	2.22	VIII C	64.0	1.52	V	16.0	1.65
III	4.9	2.72	"48"	65.0	1.55	III	19.0	1.96

* Expressed in days to LD$_{50}$.
† Longevity of selected cultures ÷ Longevity of control culture.

onstrates that hardiness (or longevity) factors accumulated concurrently with those conveying tolerance to temperature extremes. There was little difference at this temperature among the selected cultures, all of which reached the LD$_{50}$ in from 61 to 65 days.

Only at the 40°F temperature does a direct correlation exist with the mortality curve results. Because low-temperature selection had occurred at 40°F, the fact that Culture III (selected at low temperature) lived several times as long as did the control, is further corroboration of the effectiveness of selection for low-temperature tolerance. Neither VIIIC nor "48" were selected for tolerance to low temperature, so increased longevity in these cultures at this temperature could be attributed either to increased vigor (or hardiness), or to the concurrent accumulation of tolerance to a temperature extreme at which no selection was conducted (as in Culture III). The magnitude of the increase in longevity of these cultures at 40°F is impossible to ignore and difficult to explain. However, because these two cultures did not show a similar tolerance in the "mortality curve" results, it seems safe to conclude that under the conditions of the longevity tests, parasites from these cultures were able to display improved hardiness acquired as the result of selection but independently of the factors for which selection had been conducted.

Substantial improvement in all cultures at 80°F is not correlated directly with the effects of selection. At this relatively mild temperature, Culture III (selected for low temperature) displayed a substantially greater longevity than did Culture "48" which was selected for heat tolerance. Apparently, development of tolerance to extreme temperatures is independent of the accumulation of factors controlling vigor or longevity at more normal temperatures.

In work with the house fly, Varzandeh *et al.* (1954) demonstrated such independence of factors influencing biotic potential and vigor from those controlling insecticide resistance, and Crow (1957) stated: ". . . one would expect that when selection . . . is accompanied by natural selection for general fitness, the only . . . factors that would become frequent . . . would be those that cause very little reduction in fitness." He then concluded that resistance factors must be nearly neutral regarding fitness.

The effects of selection on culture hardiness and vigor can best be summarized by stating that (1) all cultures survived longer than the control at all temperatures tested, and (2) only at

<div align="center">

TABLE 8

TOLERANCE TO HIGH TEMPERATURE OF CULTURE VIIIC

</div>

Item	97°F Tolerance*		35°F Tolerance*	
	Initial	Terminal	Initial	Terminal
Culture VIII C (hours)...............	33.5	34.9	31.1	32.2
Culture I-Control (hours)............	18.9	17.1	31.0	31.0
Ratio†...............................	1.78	2.04	1.0	1.04

* Expressed in hours to LD_{50} among females.
† Selected ÷ Control.

40°F does correlation exist between the effects of selection on temperature tolerance and observed longevity. Certainly, no deleterious effects of selection were observed acting on the longevity or vigor of selected parasites.

Retention of Selected Tolerance to Extreme Temperatures

The problem of evaluating the retention of selected tolerance to extreme temperature in selected parasites was anticipated during the planning stages of the selection program. Retention evaluation required a culture in which tolerance resulting from selection was substantial, and so Culture VIIIC was formed from a portion of high-temperature selection Culture VIIIB in the F_{80} after a high level of tolerance to high temperature had been demonstrably achieved in that culture. Culture VIIIC was propagated for 18 months (through 33 generations) without any additional exposure to selection treatment.

In each generation a mortality curve was described to measure temperature tolerance. The temperature tolerance profile (figure 9) and the longevity profile (figure 10) show that Culture VIIIC retained all selected high-temperature tolerance without any loss in low-temperature tolerance, and table 8 gives specific data evaluating retention of selected tolerance. The "initial" tolerance refers to the estimate computed

by combining the mortality curve results collected in the first three generations after Culture VIIIC was formed. The terminal estimate was a similar computation based on the final six generations.

Table 8 shows that parasites selected for survival ability at 97°F demonstrated prolonged retention of tolerance at that temperature, and that this was done without affecting survival at the low temperature extreme.

Retention of selected attributes has been treated extensively in the literature. The populations developed in many selection programs regressed rapidly after termination of selection but many others demonstrated a remarkable degree of stability. For example, Crow (1954) observed no reversion to susceptibility in his artificially selected DDT-resistant strains of Drosophila after a three-year absence of selective DDT dosages. More recently, Robertson (1965) achieved stability in a line of flies selected for high numbers of sternopleural bristles. A review of the literature suggests the possibility of a correlation between retention of selected polygenically controlled attributes and the level at which selection is applied, with high stability (prolonged retention) most frequently resulting from programs in which a low level of selection was applied over a long period of time.

This correlation, if it exists, would be in agreement with the comparative sta-

bility of certain attributes observable in most species in nature. A co-adapted species under a natural field environment can survive the temperature range to which it is normally exposed, and evolutionary changes in that species usually occur in small increments over extended periods of time in response to small environmental changes or to slight geographic movements. These gradual changes working on large populations permit a condition observable in a species at any given point in time which Lerner (1954) called "genetic homeostasis."

Evaluation in the Field

Ultimate evaluation of a selected parasite strain and its effects on the host has to be made through experimentation in field situations. Such evaluation of *Aphytis lingnanensis* was planned and undertaken, but its results were heavily influenced by the importation to California, in 1956 and 1957, of *Aphytis melinus* DeBach. This parasite attacking red scale was thoroughly colonized in 1958 and thereafter (DeBach, 1959).

The field evaluation of the selected strains resulting from the program described here was attempted in 1961 and 1962 during a period when *Aphytis melinus* was spreading rapidly in California but before its ability to survive and outcompete *Aphytis lingnanensis* in interior citrus zones of California was recognized. This situation, though fortunate for the citrus industry in California, made the comparative field evaluation of the selected strains impossible. All test plots were established as planned but *Aphytis melinus*, used in some cases as a check culture, intruded into the test plots so rapidly and built up in such numbers that any evaluation of the efficacy of the selected strains of *A. lingnanensis* became impossible.

The field evaluation demonstrated only that not even the highly selected and improved *Aphytis lingnanensis* strains could successfully compete with a species, *Aphytis melinus*, inherently better adapted to the environment.

DISCUSSION

Successful selection for temperature tolerance in a beneficial insect species is of continuing interest, particularly when the selected traits are permanently fixed in the population permitting it to survive or even to thrive over a broad temperature range or under adverse temperature conditions. The fact that temperature limits were extended significantly in both directions for Culture V (and a little less dramatically for Culture VIA) permitting survival of these parasite populations over a broad spectrum of temperatures is possibly a unique accomplishment.

Although field evaluation was precluded by unforeseen events, results of laboratory evaluation indicated the vast superiority of the selected strains over the unselected control culture.

These results provide one more example of the improvement of a species for the possible benefit of man. The suggestion is inherent that similar biological control problems might be solved by artificial selection. The merits of utilizing selection techniques in seeking solutions to practical problems have been rather thoroughly argued in the literature [for arguments in favor see Sailer (1954) and Force (1967); for arguments against, see Simmonds (1963)]. There is no need to prolong that discussion, except to say that the work reported here is an apt, if unplanned, demonstration of the desirability of conducting a thorough search for all possible natural enemies of a pest species for importation before research (genetic selection or any other complex

manipulation) is conducted on natural enemies which are already established. However, these results lend substantial credence to the proposition that when pre-adapted species are unavailable, selection stands as a potent device for shaping a species into a more valuable ally after the intrinsic capabilities of that species have been carefully evaluated and the factors limiting its success have been carefully defined.

SUMMARY

Various populations of *Aphytis lingnanensis* Compere derived from a large gene pool were successfully selected for tolerance to extreme temperatures. Though irradiation techniques were employed with some of these populations, no measurable contribution could be attributed to the X-ray treatment.

Five cultures were successfully selected (through more than 100 generations) and maintained until the program was terminated. Of these, Culture III (selected for low-temperature tolerance) exhibited increased tolerance to low temperature and upon termination of selection required more than twice the time of exposure to 35°F to produce the LD_{50} than the unselected control; Cultures "48" and VIIIC were both selected for high-temperature tolerance and upon completion of the program displayed improved tolerance to high temperature by requiring more than twice the period of exposure at 97°F to produce the LD_{50} as compared to the control; Culture V which was selected for high- and then low-temperature tolerance in alternate generations exhibited increased tolerance to temperatures at both extremes. Final results showed that parasites from Culture V required 43 hours exposure to 35°F to produce the same level of mortality (50 per cent) as was obtained in the control culture in only 31 hours. At the other temperature extreme, Culture V required 31.7 hours exposure to 97°F to produce the LD_{50} while only 16.4 hours were required with control parasites.

Similarly, the temperature tolerance in Culture VI (selected at both temperature extremes in *each* generation) was improved in both directions. At the conclusion of the selection program, this culture required 57.4 hours exposure to 35°F, as compared to 31 hours for the control, to produce the LD_{50} while at 97°F, 22.5 hours were required, as compared to 16.4 for the control.

Selection contributed certain other benefits. Selection for one extreme temperature only apparently resulted in improved tolerance to both high and low extremes in Culture III. A substantial improvement in longevity of adults of all cultures was evident when tested at moderate temperatures. This indicated that hardiness (or at least that aspect contributing to longevity) of all selected cultures had been improved but in a manner that was independent of the type of selection applied.

Implications of these results are discussed.

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Tolerance to both high- and low-temperature extremes was achieved in Cultures V and VIA (successor to Culture VI). After termination of selection, Culture V parasites required 43 hours exposure to 35°F to produce the LD_{50} (which control reached in 31 hours), and they required 31.7 hours exposure to 97°F to produce the LD_{50} (reached by the control in 16.4 hours). Similarly, Culture VIA required 57.4 hours of exposure to 35°F to produce the LD_{50} (reached by the control in 31 hours) and 22.5 hours exposure to 97°F to produce the LD_{50} (reached by the control in 16.4 hours). Culture V achieved greater tolerance to high temperature and less to low temperature than did Culture VIA. Reasons are discussed.

Other observations reported: (1) The improved temperature tolerances achieved by the selected lines were apparently well fixed genetically; (2) General hardiness as indicated by adult longevity of all selected cultures was improved but in a manner independent of the type of selection applied.