LATENT GENETIC EFFECTS OF PAST SELECTION ON BLOOD FEEDING: WHY HISTORY MATTERS

by

PIPER CHARLIN KIZZIAR

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Approved: _____*Dr. William Bradshaw*_____
Primary Thesis Advisor

Conventional wisdom is that selection decreases genetic variation in populations, variation that is essential for population persistence in an ever-changing world. Basically, I find the opposite. Response to selection on biting in the pitcher-plant mosquito, *Wyeomyia smithii*, increases from 20-80%, but reverts back to the original 20% after seven generations of relaxed (not reversed) selection. At the same time, biting in the control line remains at the original 20% through 30 generations without blood feeding. Imposition of selection on biting in both lines elicits a rapid response in the previously selected line, but, importantly, not in the control line. Hence genetic variation for biting has increased, not decreased as a consequence of long-term directional selection, contrary to expectations. In short, history matters.

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Introduction

Darwinian evolution is, at its most fundamental elements, based on three fundamental observations that are widely accepted: (1) organisms vary, (2) organisms tend to increase geometrically, and (3) not all organisms survive. Darwin's insight was that during the struggle for existence, some organisms perish, while others survive and reproduce, and in so doing, pass on to their offspring the basis for their success (Futuyma 1997, Ch. 2). Darwin was ignorant of Mendel's experiments and the actual mechanics of inheritance. The melding of Darwinian evolution and Mendelian genetics was reconciled and greatly refined during the "modern synthesis" in the early to mid-20th Century by evolutionary biologists using population and quantitative genetics (Futuyma 1997, Ch. 2). In essence, evolution is any change in gene frequency in a population of organisms from one generation to the next, regardless of whether that change is the result of random sampling of the genome (genetic drift) or the result of artificial or natural selection. Artificial selection involves the imposition of reproductive success based on a single trait or group of traits; natural selection is imposed by the environment based on the genome as a whole. It is important to note that the environment imposing natural selection can be the laboratory as well as the field environment. Regardless of the agent of selection, response to that selection depends upon genetic variation underlying the trait or traits being selected (Falconer 1981, Eq. 11.2): $R = h^2S$, where response to selection (R) is the product of the heritability (h^2), a measure of the underlying genetic variation of the trait, times the strength of selection (S) applied to that trait. A corollary of this equation is that if selection is applied to a

trait in the laboratory or in nature, and there is a response to that selection, then there must be non-zero genetic variation for that trait.

It is easiest to think of selection as directional, that is towards improvement of some trait that increases fitness (ability to survive and reproduce) in populations. Examples of directional selection include selection on **geotaxis**[1] in fruit flies (Dobzhansky & Spassky 1968), dark coloration in the peppered moth during the early soot-laden days of the industrial revolution in Britain and Wales (Kettlewell 1955, 1956; Cook 2003; van't Hof et al. 2019), **warfarin**[2] resistance in Welsh brown rats (Greaves et al. 1977), organophosphate resistance in French Culex mosquitoes (Raymond et al. 2001), and improved milk yield in Brazilian dairy cows (Stefani, et al. 2018).

By its very nature, selection *selects* certain variants out of a population, presumably reducing genetic variation and leaving the population less able to respond to selection. The problem then arises as to what maintains genetic variation when confronted by selection. One of the answers relates to trade-offs between the components of or elements contributing to survival and reproduction (fitness). In the case of fruit flies, selection on geotaxis also resulted in correlated differences in body size, eye size, testis color, and wing venation (Dobzhansky & Spassky 1968). When selection was relaxed (not applied), geotaxis, as well as its correlated traits, tended over several generations towards the pre-selection values. Similarly, selection on dark coloration in industrial moths also resulted in altered structure as well as color of wing

[1] Movement in response to gravity: positive = upwards; negative = downwards
[2] Rat poison that causes internal bleeding when ingested.

scales (van't Hof et al. 2019). When industrial pollution abated in Wales and England, the frequency of dark, soot-colored moths declined and that of moths resembling light tree bark with lichens increased (Cook 2003). Increased warfarin resistance in brown rats also can result in excess blood clotting within the circulatory system (Bishop 1981). Organophosphate resistance in French mosquitoes resulted in a loss of fitness in the absence of the pesticide (Lenormond & Raymond 2000; Raymond et al. 2001). Finally, improved milk yield in Brazilian cows resulted in shorter milk-producing years and reduced udder health (Stefani et al. 2018). The common theme here is that strong selection on a single trait incurs a correlated cost so that once artificial selection is relaxed, natural selection returns not only the selected but also the correlated traits towards the pre-selection values. Hence, a gene or suite of genes underlying a given trait can affect multiple other traits (pleiotropy) that may have an opposite effect on fitness (antagonistic pleiotropy). In the presence of strong selection on one trait, the value of that trait becomes the over-riding definition of fitness, with deleterious pleiotropic effects, whether visible or not, having a negative, but subsidiary effect on fitness. When selection is relaxed, the tendency is towards a renewed balance between the trait previously under selection and its pleiotropic effects.

Herein, I first confirm that relaxation of selection on blood feeding in a mosquito results in reversion to the pre-selected value and, second, I determine whether the prior history of blood feeding lies genetically latent in the selected population. This question is relevant because, if selection history matters, then, by extension, genetic variation that is not immediately apparent, can affect both the likelihood of acquiring a disease as well its severity and ultimate prognosis of that disease. Does history matter?

The subject of my research is the mosquito *Wyeomyia smithii* that lays its eggs and completes its pre-adult development only within the water-filled leaves of the purple pitcher plant, *Sarracenia purpurea* (Bradshaw 1983). Adult females in southern populations produce their first batch of eggs without taking a blood meal (biting). To produce a second or subsequent batch, they require a blood meal, but only a fraction of the population does so and this fraction is a heritable trait (genetically variable), capable of responding to selection (Borowczak 2017; Bradshaw et al. 2018). Borowczak (2017) selected on propensity to bite an anaesthetized rat in a Florida population (Figure S1), resulting in an increase of biting from 20-80% of females over 18 generations (Fig. 1).

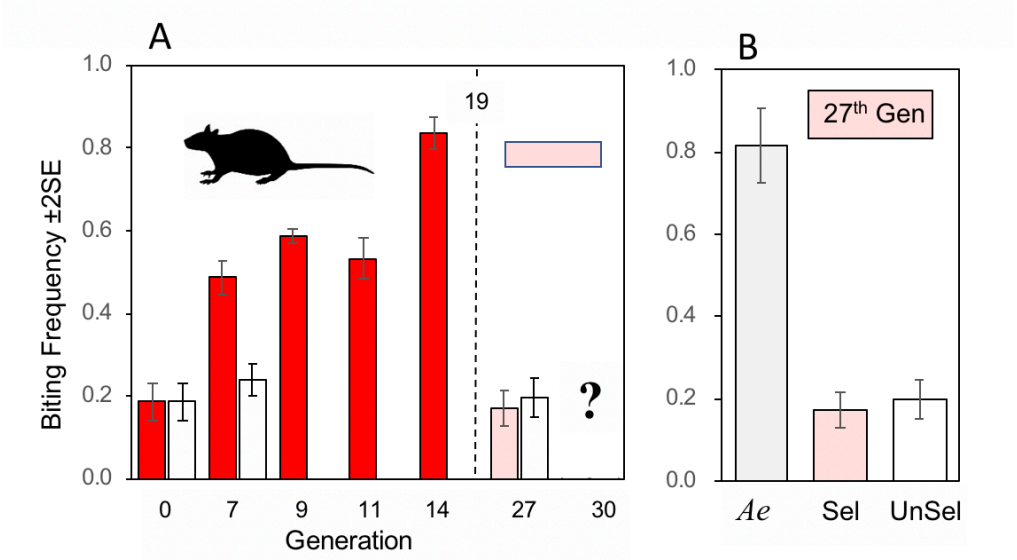

Fig. 1. Biting history. **A.** Frequency of biting on a rat in the original selected line (red), the nonbiting control line (white), and on pledgets soaked in defibrinated sheep blood (pink). Dashed vertical line marks end of blood feeding on a rat and maintenance of the selected line from non-biters (pre-biters). **B.** Frequency of biting in generation 27 from blood-soaked cotton pledgets in a viciously biting *Aedes sierrensis* (*Ae*), in the selected line from 1A (pink), and in the unselected control line (white). Error bars represent ±2 standard errors. The first step is to confirm or refute in generation 30 (?) the results in generation 27. Compiled from Borowczak (2017) and Kirsch (2019).

Borowczak (2017) maintained a control line that was never exposed to a host or had any opportunity to bite. Propensity to bite in the unselected control remained around 20%. After the 19th generation, blood meals were no longer offered to the selected, blood-feeding line and both the selected and control lines were reared with no further (relaxed) selection through the 30th generation based only on raisins as a carbohydrate source for the adults. At the 27th generation (8th generation of relaxed selection) to test for propensity to bite in that generation, Kirsch (2019) used a cotton dental pledget soaked in defibrinated sheep's blood. He found that the propensity to bite in the selected line had converged on the unselected control line (Fig. 1). Importantly,

since Kirsch (2019) changed the host from a rat to a pledget, he used an avidly biting local mosquito, *Aedes sierrensis*, to "truth" the effectiveness of the pledget as an artificial host. Kirsch found that 80% of the *Ae. sierrensis* took a blood meal from the pledget, confirming that the blood-soaked pledget served as a viable "host" in lieu of a rat.

Given the above results, I ask two specific questions:

(1) Are Kirsch's results repeatable? Can I confirm or refute convergence of the selected with the control line?

(2) Given that the selected and control line converge on the same value, has previous selection on biting increased, decreased, or had no effect on the genetic variability underlying propensity to bite as compared to the unselected control?

The latter question addresses the crux of the issue. Selection, whether natural or artificial, selects from an assemblage of variable, genetically determined traits, and by virtue of the very process of selection itself, should reduce underlying genetic variation. In the present case with blood feeding in *Wyeomyia*, at generation 30:

(A) a decrease in genetic variation in the selected, biting line as compared to the unselected control, would lead us to conclude that response to previous selection had reduced from intermediate to low frequencies the non-biting **alleles**[3] in the population.

[3] Alleles are alternate forms of the same gene, capable of being inherited separately. In a population of organisms, there may be many alleles of varying frequency for any given gene.

(B) an increase in genetic variation in the selected, biting line as compared to the unselected control, would lead us to conclude that response to previous selection had increased the biting alleles from low to intermediate levels.

I therefore imposed secondary selection on blood feeding, starting at generation 30, to determine whether the response to selection for biting were lower (A) or higher (B) in the biting than in the control line.

Materials and Methods

Larval Rearing:

All stock populations were kept in 150 x 25 mm Petri dishes containing a larval medium of 1.08 x 10^{-1} mg/mL tetracycline water and 2 mL of larval food. Larval food consisted of four parts Geisler Guinea Pig Feed (Sergeant's Pet Care Products) and one part San Francisco Bay Brand freeze dried brine shrimp. The first generation of experimental larvae was selected randomly from stock populations and moved from the **short-day room**[4] to the **long-day room**[5] to induce development. Every Monday, Wednesday, and Friday, pupae were collected and placed in a transparent cup filled with up to 50 pupae in each. Larvae were reared in tap water previously conditioned in 38 L polycarbonate carboys at room temperature exposed to northern light; pupae were reared in Crystal Geyser spring water (Walmart). After two to three days, the pupae cups were placed in a cage containing a *Sarracenia purpurea* leaf for oviposition and pesticide-free raisins as a carbohydrate source. Eggs were collected three times a week in 150 x 25 mm Petri dishes; resulting larvae were reared in the short-day room. Hatch were collected five days after egg collection and larvae were placed in 150 x 25 mm Petri dishes containing the larval medium, with 35 larvae per dish.

[4] Room conditioned to maintain diapause. The short-day room is programmed for 21°C, 0% humidity, and 10 hours of light per day.

[5] Room conditioned to end diapause and stimulate maturation. The long-day room is programmed for >80% humidity, 18 hours of light per day, and a daily temperature sine wave between 15°C and 32°C each day that lags behind the light cycle by 3 hours.

Randomization Procedure (Histogram Method)

When hatch of a given generation in the selected or control line exceeded 5,000, the line was thinned or experimental animals removed using a "histogram" method. Dishes from each oviposition date were stacked together and the stacks arranged sequentially by oviposition date, forming a three-dimensional histogram of dishes. For example: (1) To thin by 2/3, the first dish on the first date of oviposition was retained to maintain the line, the next two dishes were removed and discarded or used for experiments, the fourth dish retained to maintain the line, and so on. (2) To extract experimental larvae equal to $1/7^{th}$ from either the selected or control line, the 7^{th} dish in the histogram was removed and used for experiments, then the 14^{th}, 21^{st}, and so on. The remaining $6/7^{th}$ of the histogram was retained as stock for the selected or control line. Experimental larvae were pooled in a large pan and sorted into dishes of 35. Neither any larvae removed for thinning or for experiments or their progeny were ever returned to the selected or control line.

Blood Feeding:

Experimental animals were offered a blood meal three times a week on a consistent Monday, Wednesday, Friday schedule from 12:00 to 14:00. I used Kirsch's (2019) blood feeding apparatus that uses warm defibrinated sheep's blood (Hardy Diagnostics) and dry ice to simulate a live animal (Figure 2). Five mL of blood was heated to 45°C in a water bath. The 50 mL syringe in the apparatus was filled to the 5 mL mark with one or two pieces of dry ice, generating a stream of cold CO_2 that was heated in a water bath to 45°C. Using a needle attached to the heated stream of CO_2, I impaled a cotton pledget, dipped the pledget into the warmed blood and hung the blood-

soaked pledget through the top of the cage with a 5 cm gap between the top of the pledget and the top of the cage. The warm, blood-soaked pledget emanating CO_2 was left in each cage for 20 minutes. I reheated the water bath and blood to 45ºC before inserting into the next cage, repeating the process until the mosquitoes in all cages had the opportunity to blood feed. A mosquito was scored as blood feeding if there was an observed kink in her **labium**[6] and blood was visible in her abdomen. I used an aspirator to transfer to a separate cage each and every mosquito that took a blood meal and recorded the number of biters.

[6] Outer sheath of the proboscis that bends outwards (kinks) as the internal stylet is inserted into the host.

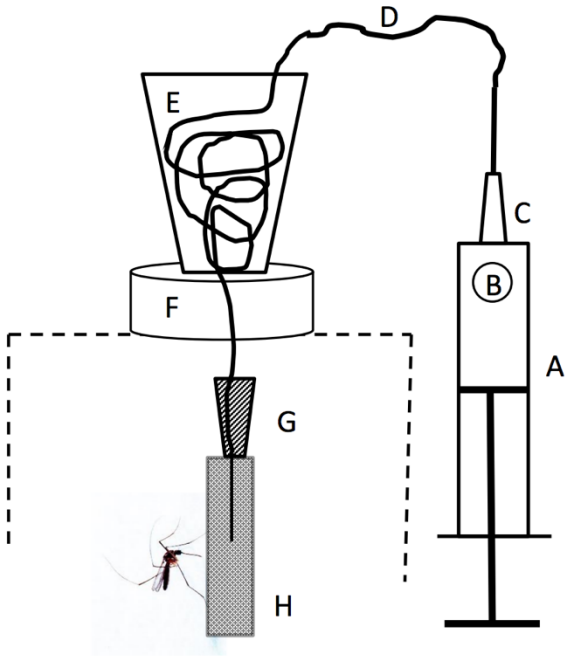

Figure 2. Apparatus for infusing warm CO_2 through blood-soaked cotton pledgets (Kirsch 2019). **A**. 50 ml syringe; **B.**1-2 chunks of dry ice ca. 1 cm diameter; **C.** 20 gauge 1.5" = 3.8 cm needle inserted into **D.** 1.67 mm diameter poly tubing. **E.** Poly tubing coiled inside 45 ml cup; **F.** Foam insulator ca. 1.5 cm thick, 4cm diameter; **G.** 18 gauge, 1.5" = 3.8 cm needle with the end of the poly tubing glued into it; **H.** Cotton roll dental pledget soaked in warm defibrinated sheep blood. Dashed line, top of mosquito cage.

Selection on Blood Feeding:

To determine if genetic variation in propensity to bite in the previously selected 80% biting line persisted through the 12 generations without selection (Fig. 1A), I determined propensity to bite in the F30 generation of both the previously selected and control lines. I then imposed renewed selection on blood-feeding for three subsequent generations in both lines. Using the histogram technique, 1260 mosquito larvae in the F29 generation were sampled from the stock populations of each line and allowed to develop to adults and lay eggs using the larval rearing procedure. Data were not

collected during this generation, as developing the populations through the first generation simply ensured that both populations were the same age under the same conditions.

The 30th and the three subsequent generations were selected on for blood feeding. Using the standard larval rearing procedures, larvae developed into pupae and adults. During each generation, eggs were only collected from the biting cages, whose hatch was reared in the short-day room to synchronize development. After synchronization, larvae were reared to adults for the next iteration of selection. The **exuviae**[7] were collected from the pupal dishes and sexed to determine the percentage of female biters in each line from each generation of selection.

[7] Remains of the exoskeleton that a mosquito sheds when developing from a pupa to an adult. Males and females have different morphology shown in the exuviae, allowing one to determine the number of males and females.

Results and Discussion

Results from Borowczak (2017) showed that after 14 generations of initial selection, the incidence of biting in the selected line increased from 18% to 84% (Fig. 2A). Selection was continued through the 19th generation, but incidence of biting was not determined from the 14th to 19th generation (Borowczak 2017). After the 19th generation, selection ceased and both the selected and control lines were maintained without access to a host through the 30th generation with adults having access only to raisins. After eight generations (20th-27th) without exposure to a host, incidence of biting in the selected line declined to the ancestral level, while biting in the control line remained at the ancestral level (Kirsch 2019). I confirmed Kirsch's results in the 30th generation (Fig. 3A).

Since the host was switched from a live rat to a blood-soaked pledget, the question arises as to whether the decline in biting in the selected line after blood meals were no longer offered was due to an actual genetic decline in propensity to bite, or to the exposure to a novel host. Two factors indicate that the low incidence of biting by the selected line was due to relaxed selection and not host specificity. First, the high incidence of biting in *Aedes sierrensis* exceeded 80% on the blood-soaked pledget (Fig. 1B), demonstrating that the artificial host was not inhibiting a naturally avid-biting mosquito. Second, the incidence of biting in the unselected line did not decline below the ancestral level in either the 27th or 30th generation (Fig. 3A), demonstrating that access to a pledget, in the absence of a rat, did not elicit a lower propensity to bite. I conclude that initial response to selection resulted in an increase from 20-80% biting females due to artificial selection and that the decline back to 20% in generations 27

and 30 was due to relaxed selection itself, and not to exposure to a novel host.

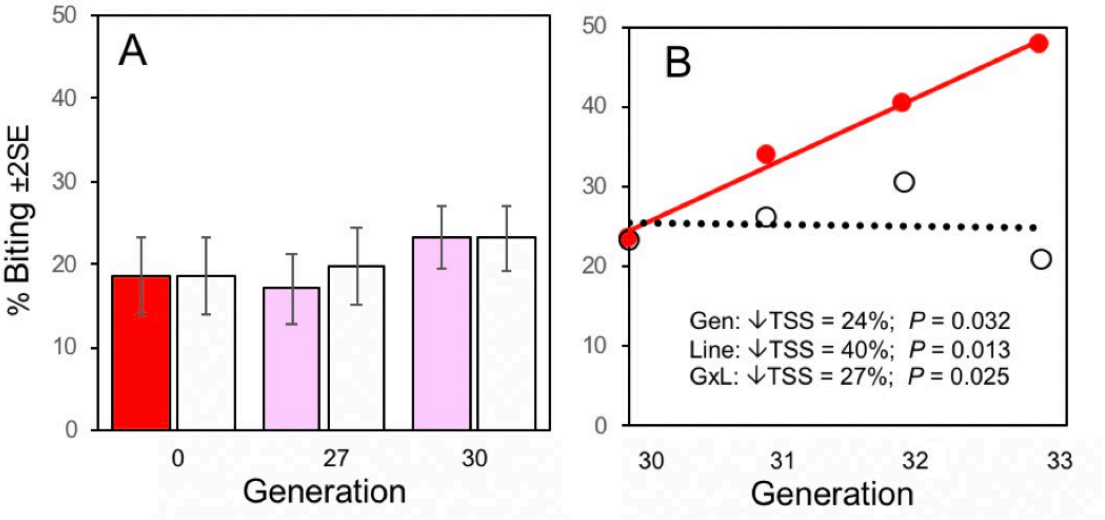

Figure 3. **A.** Biting propensity in the selected line (red or pink) and the unselected control line (white) before selection (Generation 0), after 19 generations of selection followed by eight (Generation 27) or 11 generations of relaxed selection (Generation 30). **B.** Response to renewed selection for biting starting in Generation 30 on both the previously selected line (red) and the previously unselected control line (white). ↓ TSS = percent reduction in total sum of squares from ANCOVA (Table S1).

The convergence of biting in the control and selected lines on a strictly adult carbohydrate diet prompted the question: Does this convergence to 20% biting also reflect a convergence in genetic propensity to bite; or, does the selected line potentially harbor latent genetic variation that could respond to renewed selection? The results in Figure 3B show that previous selection for blood feeding has retained genetic variation enabling the formerly selected line to respond strongly to renewed selection as compared to the control line. There had been a convergence in biting behavior but not genotype: latent genetic variation inherited from past selection persisted, and actually increased during relaxed selection and despite reversion to the ancestral biting levels. History matters.

Borowczak (2017) successfully selected for increased biting starting with an initial population size of 14,000. My results (Fig. 3B) show that an initial population of 1,000 in the control line fails to elicit a significant response to selection on blood feeding. These results emphasize the importance of large population sizes, especially when developing selected lines for traits with low initial heritability.

Underlying genetic liability can also be an important concept in an historical context. Inherited resistance to mosquito-borne diseases greatly influenced the African slave trade and subsequent racism towards African Americans. Malaria is endemic to many parts of Africa, and selection over hundreds of generations of exposure to malaria has resulted in variation among those of African descent in their liability for malaria infection (McNeill, 2010, Chapter 2). This variation is largely attributed to inherited physiological traits such as **sickle cell anemia**[8] and **thalassemia**[9] that inhibit the malaria parasite's ability to infect red blood cells (Stevenson & Riley 2004). Additionally, while the mechanism of resistance against yellow fever is still unknown, statistical studies and observational evidence of yellow fever epidemics point to inherited resistance against yellow fever in those of African descent (Kiple & Kiple 1980; McNeill, 2010, Chapter 7). The difference in inherited immunity against malaria and possibly yellow fever between those of African and European descent, leading to Europeans and Americans willing to pay the expense of transporting African laborers to plantations in the Americas rather than establishing plantations in Africa at the risk their own survival. This practice ultimately backfired as the very diseases, against which

[8] Inherited disorder that causes red blood cells to contort into a sickle shape. Both carriers and those homozygous for the mutation are resistant to malaria.
[9] One of the most common inherited disorders. The mutation leads to decreased levels of hemoglobin, and both homozygous and heterozygous individuals show resistance to malaria.

Africans had inherited immunity, were also transported across the Atlantic. Nonetheless, African labor became increasingly more valuable. Even as slavery became a more unpopular institution in the United States, Southern physicians justified the practice by citing the resistance of African slaves against mosquito-borne diseases and arguing that they were members of a servile race, better suited to labor in plantations (Spielman and D'Antonio, 2001, Chapter 4). While Africans had undergone centuries of selection against mosquito-borne diseases, the resulting inherited resistance, itself, ultimately led to an immense amount of suffering for those caught in the slave trade and to subsequent racism against African Americans, even after the abolition of slavery. History matters on both evolutionary and sociological time scales.

Conclusions

Whether provided with a natural host (rat) or an artificial host (blood-soaked cotton), propensity to imbibe a blood meal (bite) in a southern population of *Wyeomyia smithii* is polymorphic and responds to positive selection. When selection is relaxed, propensity to bite rapidly declines and converges with the unselected control line, both lines of which hover around 20% for more than 10 additional generations. When selection is reimposed on both selected and control lines in generation 30, the original selected line responds rapidly to renewed selection, while, importantly, the unselected control line exhibits no response. I therefore conclude that initial selection had increased, not decreased genetic variation for blood feeding. I also conclude that there are trade-offs between blood-feeding and other components of fitness, since genes regulating propensity to bite have a low, but consistent persistence, even in the absence of blood feeding.

Appendix

Table S1: ANCOVA (Sall, et al., 2005, pp. 354-360) of percent biting with generation of renewed selection as the covariate and past selection lines (selected for biting generations 1-19 vs. control nonbiting generations 1-19) as treatments (Fig. 3).

Source of Variation	DF	Sum Sqs	Mean Sq	F	P	%↓TSS
Generation	1	147.46	147.46	10.51	0.032	23.624
Line	1	250.88	250.88	17.89	0.013	40.194
G x L	1	169.74	169.74	12.10	0.025	27.195
Error	4	56.10	14.03			
Total	7	624.18				

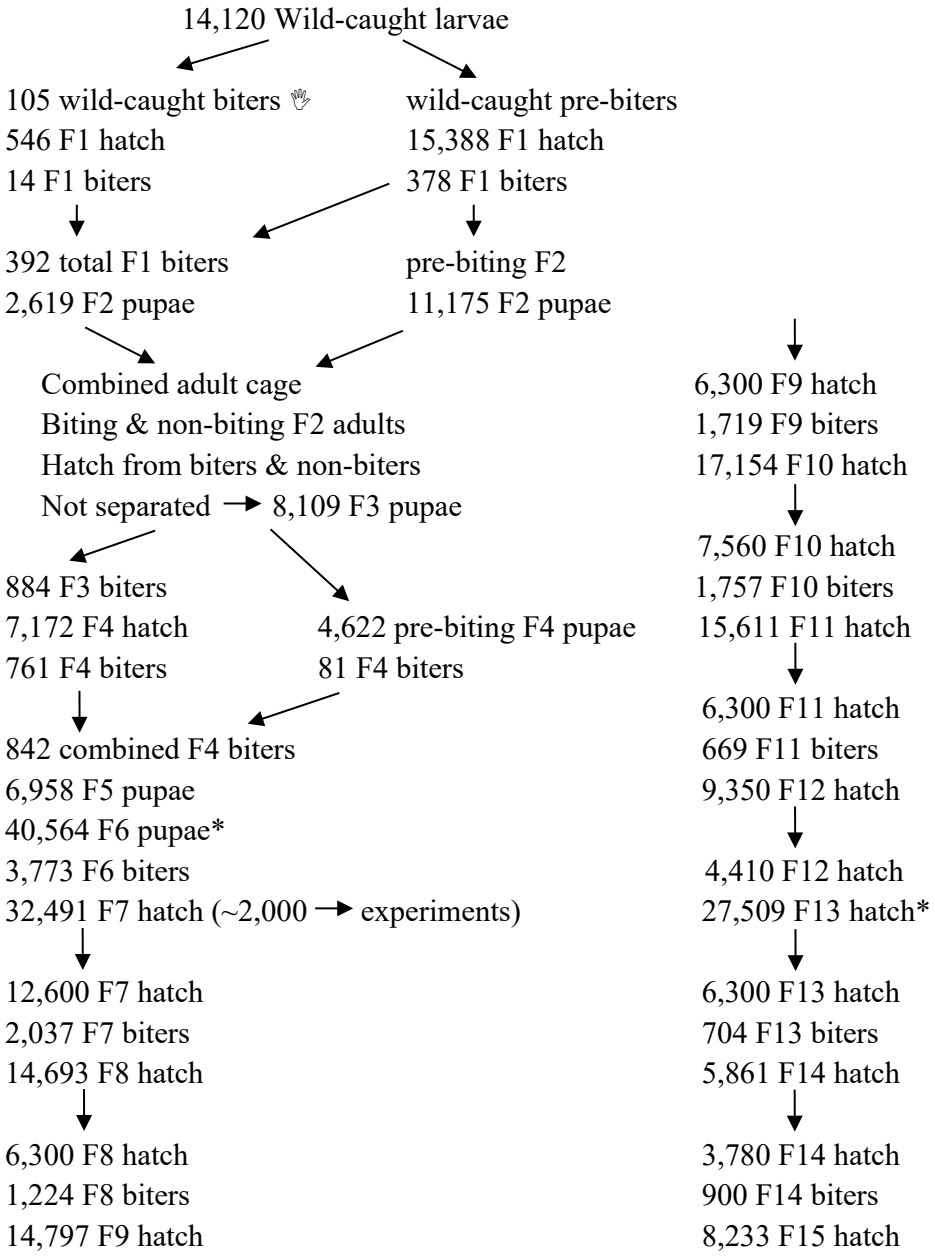

Figure Sl. Generation and maintenance of the line selected for avid biting. The complex manipulations in early generations are due to the fact that, initially, total hatch from biting females was not sufficient to generate a cohort replacement rate (Ro) greater than 1.0. Consequently, until Ro 1.0 was reached, hatch from biting females was comingled with hatch from females of the same generation that had not taken a blood meal (pre-biters). Until Ro was 21.0, all hatch from blood feeding females were used to generate the selected line; thereafter, hatch in excess of Ro>l .0 were available for experiments. human blood source; all other generations used a rat as a host;

*Reared through one generation without a blood meal to augment the selected line.

Compiled from Borowczak (2017).

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