EFECTO DE LA VARIABILIDAD GENÉTICA EN EL DESEMPEÑO DEL PARASITOIDE *Trichogramma pretiosum* WESTWOOD (HYMENOPTERA: TRICHOGRAMMATIDAE)

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PRESENTADA POR:
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en la preparación académica y profesional.

COMPENDIO

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POR

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Palabras clave: *Trichogramma pretiosum*, líneas puras, parámetros poblacionales, tasa sexual, tamaño, variabilidad genética, desempeño, marcadores moleculares.

El objetivo de esta investigación fue evaluar el efecto de la variabilidad genética en el desempeño de *T. pretiosum.* Para lo cual se desarrollaron tres experimentos.

En el primer experimento se compararon 26 líneas puras de *T. pretiosum* mediante sus parámetros poblacionales, fecundidad, tamaño y tasa sexual. Esta información se utilizó y complementó para elegir y combinar

algunas líneas puras y evaluarlas, ya sea por distinto número de generaciones o por una conformación distinta de líneas. Las poblaciones resultantes o líneas fueron: (1) la "genéticamente variable" (resultado de la combinación de 26 líneas puras) con dos generaciones; (2) la "genéticamente variable" con seis generaciones; (3) la "genéticamente variable" con 17 generaciones; (4) la línea "rm alto", resultado de la combinación de dos líneas puras con alta tasa de incremento rm y fecundidad a las 48 h; (5) línea "rm baja", combinación de dos líneas puras con baja tasa de incremento rm y fecundidad a las 48 h, finalmente; (6) "reproducción en masa", compuesta por una población comercial. En este experimento no se encontró diferencia significativa entre la “genéticamente variable" con dos generaciones y "rm alto” (líneas 1 y 4) a las 48 h. Sin embargo estas líneas con mejor desempeño fueron distintas de las restantes 4 líneas (2, 3, 5 y 6) a 48 h. En la fecundidad total todas las líneas "genéticamente variables" y "rm alto" (1, 2, 3 y 4) fueron significativamente distintas a la "rm baja" (5). Se encontró que usar hembras mayores de 6 días, en las poblaciones fundadoras, es contraproducente por el incremento de machos en los días subsecuentes, lo que incrementa el costo de producción.

En el segundo experimento se estudió el efecto de la variación genética en la relación al tamaño-fecundidad en las líneas de *T. pretiosum* puras. Para lo cual se utilizó 26 líneas puras (altamente entrecruzadas) del experimento anterior, y sus parámetros se usaron para crear nueve poblaciones experimentales. Las primeras seis poblaciones o líneas puras fueron: dos de alta fecundidad (líneas 29 y 43), dos con baja fecundidad (líneas 2 y 53) y dos

con fecundidad intermedia (líneas 1 y 9). Una séptima población se creó mediante la mezcla de las seis líneas anteriores (1, 2, 9, 29, 43 y 53). La octava y novena poblaciones se combinaron (26 líneas puras, una con 2 y otra con 40 generaciones). Los resultados indicarón en las seis líneas puras (de manera individual) una fuerte correlación positiva entre el tamaño de la hembra y la fecundidad, pero se observó diferencias relacionadas a su carga genética, por lo que las líneas más fecundas fueron significativamente más grandes. Además se observó una relación constante en los ensayos realizados con más de 2 años de separación (78 generaciones) en las líneas puras. Por otro lado, la población séptima, creada como una mezcla de las líneas altamente endogámicas, funcionó como una población verdaderamente "promedio", y se perdió la correlación entre el tamaño de avispa y la fecundidad. En la comparación de las poblaciones octava y novena, contrariamente a lo esperado, se encontró que el desempeño de una población genéticamente variable (creado a partir de 26 líneas puras diferentes) no disminuyó en tamaño como resultado de 40 generaciones de cría en laboratorio, pero si en la fecundidad en donde se encontró diferencias significativas.

En el tercer experimento se evaluaron los parámetros poblacionales fecundidad y tasa sexual de líneas puras y genéticamente variables de *T. pretiosum.* Con base en los experimentos previos se eligieron dos líneas puras con bajo y alto desempeño (2 y 43 respectivamente), con 95 generaciones aproximadamente y dos genéticamente variables, producto de la combinación de 26 líneas puras con 2 y 40 generaciones bajo las mismas condiciones

climáticas, alimenticias y de hospedero. Los resultados mostraron que tanto en los parámetros poblacionales Ro, T, λ y rm, al igual que la fecundidad, la línea genéticamente variable con dos generaciones presenta los mejores resultados, seguido de la genéticamente variable con 40 generaciones, después se ubicó la línea 43 y por último a la línea pura 2. A las 48 horas, no existen diferencias significativas en la fecundidad y la proporción sexual entre las líneas genéticamente variables y la línea pura 43, relación que cambia en la proporción sexual total, donde se observó un mayor número de machos en las líneas genéticamente variables que en las lineas puras.

ABSTRACT

The objective of this research was to evaluate the effect of genetic variability in *Trichogramma pretiosum* in order to improve its performance. Three main experiments were performed to achieve this goal.

In the first experiment 26 *Trichogramma pretiosum* isofemale lines were compared through their population parameters, fertility, size and sex ratio. This information was used to choose and hybridize isofemale lines, and to evaluate them by different number of generations or by a different conformation of lines. The resulting populations were: (1) the "genetically variable" (consisting of 26 lines) interbread by two generations; (2) "genetically variable" with six generations; (3) "genetically variable" with 17 generations; (4) "rm high", combining two inbred lines chosen for their high intrinsic rate of increase (rm) and fecundity at 48h; (5) "rm low", combining two inbred lines selected on the basis of a low (rm) and fecundity at 48h, finaly; (6) "mass rearing ", consisting of a commercial population. No significant differences between "genetically variable" with two generations to "rm high" (lines 1 and 4) at 48 h were found in this experiment. However, these better performing lines were different to the remaining four lines (2, 3, 5 and 6) at 48 h. Total fertility in all lines "genetically variable" and "rm high" (1, 2, 3 and 4) were significantly different from the "rm

low" (5). We found use females over 6 days old in founder populations are counterproductive, because males production increased in the subsequent days, which increases the production cost.

In the second experiment the effects of genetic variation on the relationship between size and fecundity in inbred *Trichogramma pretiosum* lines were studied. Twenty-six genetically different, but highly inbred, lines were used to create nine experimental populations. Six lines were selected, two with high (lines 29 and 43), two with low fecundity (lines 2 and 53), and two with intermediate fecundity (lines 1 and 9) and each used to represent one of six highly inbred populations. A seventh population was created by mixing the six previous lines, and two further populations were created as a mixture of all 26 inbred lines, which were then reared for either 2 or 40 generations. Across the highly inbred lines there was a strong positive correlation between female size and fecundity, and by controlling for this relationship, we revealed the effects of genetic variation, lines with higher fecundity were significantly bigger. The performance of the highly inbred populations formed a consistent hierarchical ranking in trials performed over 2 years (78 generations) apart. In comparison, the population created as a mix of the highly inbred lines, performed as a truly "average" population, and the correlation between wasp size and fecundity was lost. Contrary to expectations, we also found that the performance of a genetically variable population (created from 26 different inbred lines) did not decrease as a result of 40 generations of mass rearing, but fecundity changed, yielding significant differences.

In the third experiment we evaluated the population parameters, fecundity and sex ratio of inbred and genetically variable *T. pretiosum* lines. Based on previous experiments we chose two inbred lines with low and high performance (2 and 43 respectively) with approximately 95 generations and two genetically variable, the product of the combination of 26 isofemale lines with 2 and 40 generations under the same climatic, food and host conditions. The results show that for both the four population parameters (Ro, T, λ and rm), and lifetime fecundity, had the best performance in the genetically variable line with two generations followed by the genetically variable population with 40 generations, then isofemale line 43 and finally the isofemale line 2. At 48 h no significant differences were observed in fecundity and sex ratio between the genetically variable populations (both 2 and 40) and the isofemale line 43. This relation changes in the lifetime sex ratio, where we observed a higher number of males in genetically variable lines than isofemale lines.

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INTRODUCCION

La reproducción de *Trichogramma* es de gran trascendencia en México y el mundo, por el número de individuos que son reproducidos y liberados periódicamente. De los 58 centros de reproducción de organismos benéficos en todo el país, 59% reproduce insectos y de estos centros el 91% distribuye y comercializa alguna especie de parasitoide para ser liberado en programas de control biológico, entre los que se destaca la especie de *Trichogramma pretiosum* (Arredondo Bernal, 2010). Al incrementar en condiciones artificiales cualquier organismo biológico, se influye en la variabilidad original de la población (Nunney *et al.*, 2002). Esta variabilidad está ligada a todos los atributos reproductivos como fecundidad, tasa sexual, capacidad de búsqueda, tamaño, entre muchos otros.

En programas de control biológico clásico (introducción de especies) o por aumento (liberaciones masivas) la variabilidad genética promueve la adaptación del enemigo natural al ambiente (Chassain & Boulétreau, 1991; Hopper *et al.*, 1993; Wajnberg, 2004). Sin embargo mantener la variabilidad genética durante el proceso de reproducción de *T. pretiosum* en cautiverio es difícil. Gran cantidad de individuos se necesitan en el proceso de control y, al incrementar el número de individuos de una población, también afectamos la

calidad de la misma (Nunney, 2003). Una pérdida de variabilidad puede darse debido a que algunos individuos están mejor adaptados a las condiciones artificiales que otros, promoviendo la selección y/o eliminación de algunos alelos (Bartlett, 1984; Allendorf, 1986; Nunney *et al.*, 2002), y esta eliminación de variabilidad puede conducir a un bajo desempeño en campo (Woodworth *et al.*, 2002; Frankham, 2005). Manejar una población numerosa de insectos, con variabilidad genética, sin un flujo genético constante, por un número alto de generaciones, incrementa las posibilidades de seleccionar ciertos alelos y una acelerada adaptación a las condiciones artificiales (Woodworth *et al.*, 2002). Las implicaciones económicas de las actividades realizadas para detectar, colectar, transportar, determinar, y cuarentenar poblaciones libres de hiperparásitos y enfermedades, impide una renovación constante de las poblaciones en los laboratorios de cría artificial.

La pérdida de variabilidad puede ser controlada al menos de dos formas, la primera, subdividiendo una población natural recién colectada del campo, y posterior a las reproducciones necesarias para su incremento, reconstituir la variabilidad inicial, uniéndolas antes de su liberación en campo (Margan *et al.*, 1998). Una segunda opción es crear líneas puras, a través de la reproducción hermanada, por algunas generaciones y eligiendo aquella que combine un desempeño óptimo en ambos tanto en reproducción masiva, como en campo (Roush & Hopper, 1995; Kalyebi *et al.*, 2005; Nunney, 2006). Producir líneas puras a partir de una población recién colectada en campo asegura la selección aleatoria de sus alelos, y los mantiene constantes, ya que son homocigotos

para cada característica, al no tener variabilidad que perder, no tienen cambios en el tiempo (excepto a través de mutaciones). Un ejemplo de su efectividad lo podemos observar en el uso de *Encarsia formosa*, con partenogénesis telitoquia, 100% homocigota, usada con éxito en Europa para el control de la mosquita blanca en invernaderos desde 1930 (van Lenteren *et al.*, 1997).

Para determinar el desempeño de las líneas puras y poblaciones, y elegir aquella que se adapte mejor a las condiciones de laboratorio, se han utilizado distintos métodos estadísticos. Las tablas de vida han sido consideradas como una buena herramienta para estimar parámetros de crecimiento, desarrollo y reproducción (Lotka, 1907; Lewis, 1942; Leslie, 1945; Southwood, 1978; Bellows Jr. *et al.*, 1992; Maia *et al.*, 2000; Badii & Castillo, 2009). Por otro lado existen otros trabajos relacionados con la evaluación de la calidad en parasitoides en los que se ha usado la fecundidad, tamaño, longevidad y tasa sexual (Waage & Ming, 1984; Kazmer & Luck, 1991; Antolin, 1999; Hoffmann *et al.*, 2001).

El presente trabajo utilizó líneas puras de *Trichogramma pretiosum* como una forma de entender el desempeño de este parásito en condiciones de laboratorio. El propósito final de estos experimentos fué proporcionar una herramienta útil para laboratorios de reproducción masiva y una evidencia de los beneficios de utilizar líneas puras, evitando la domesticación y manteniendo la variabilidad genética.

OBJETIVOS

Objetivo general

Estudiar el efecto de la variabilidad genética en el desempeño relativo de *T. pretiosum*

Objetivos particulares

- Establecer el desempeño relativo entre líneas puras de *T. pretiosum*
- Comparar el desempeño de una población genéticamente variable (compuesta por 26 líneas puras), con poblaciones compuestas con una o dos líneas puras.
- Evaluar el desempeño de poblaciones genéticamente variables en relación al número de generaciones.
- Determinar la relación entre la variabilidad genética el tamaño y la fecundidad.

REVISION DE LITERATURA

Aspectos generales de *Trichogramma*

Sistemática

En cualquier programa de control biológico, así como en la mayoría de los estudios científicos en donde se involucra insectos, proveer una identificación correcta es el primer paso para un programa exitoso (Heraty, 2004). Sorprendentemente y a pesar de que el género *Trichogramma* ha sido grandemente estudiado desde hace más de cien años y utilizado intensivamente por muchos países como México, permanece pobremente conocido taxonómicamente, una explicación a este fenómeno es acompañada por la gran cantidad de especies y a la dificultad en la identificación, en donde obligatoriamente se usan los machos y se preparan manualmente complejas laminillas, debido a su diminuto tamaño (Noyes, 1978; Pinto, 1998; Platner *et al.*, 1999).

Sistemática clásica

Uno de los principales trabajos que resumen la información sobre la sistemática clásica de *Trichogramma* en este continente, es la realizada por Pinto en 1998, en su publicación sobre la sistemática de las especies de

Trichogramma de Norte América, se incluye lo más importante en publicaciones recientes, biología, terminología en anatomía y estructura, clasificación, filogenia, claves. El género *Trichogramma* está representado por 180 especies en el mundo, 68 para el continente Americano. Estas pequeñas avispas no exceden los 0.7 mm de longitud. Se distinguen por poseer el abdomen sin una constricción entre el mesosoma y el metasoma. Las hembras y machos pueden ser separados por la morfología de las setas antenales; plumosas y de mayor longitud en machos. Las antenas de los machos generalmente con flagelos de dos a nueve segmentos (usualmente de 3-7), incluyendo 1 o 2 anillos, los segmentos funiculares de 0-2 y clava con 1-5 segmentos. Las alas pueden ser angostas y delgadas o ausentes y atrofiadas, comúnmente anchas y redondeadas apicalmente, con vena postmarginal ausente o muy cortas, se observan setas discales en alas anteriores y posteriores en conformaciones lineales. Tarsos de tres segmentos. Su color varía de amarillentos a café muy oscuro, en ocasiones con dos tonalidades, aunque pueden encontrarse en colores anaranjados o rojos. Algunos ejemplares presentan ojos rojos (Pinto, 1998).

Biología

Trichogramma como agente de control biológico

Parasitoide estricto de huevecillos de hábitos solitarios, gregario en algunas ocasiones. *T. pretiosum* es una especie ampliamente usada en muchos países para el control biológico de Lepidópteros, tant palomillas como

mariposas (Stinner, 1977) y es la más comúnmente aplicada en programas de control biológico en México (Rodriguez-del-Bosque & Smith, 1997; Rodríguez-del-Bosque & Arredondo, 1999; García-González *et al.*, 2011). Algunos otros hospederos conocidos pertenecen a los órdenes Coleóptera, Díptera, Hymenoptera y Neuróptera (Pinto *et al.*, 1986). Por su capacidad de parasitar distintos órdenes, es considerada una especie altamente polífaga, lo que en teoría la hace atractiva para el uso en control biológico.

Distribución y hospederos de *T. pretiosum*

Es la especie mayormente distribuida en Norteamérica, encontrada en hábitats agrícolas y de disturbio. Pinto (1998) reporta 240 hospederos para esta especie, pertenecientes a solo dos órdenes: Neuróptera en Chrysopidae de especie indeterminada y Lepidóptera en especies como *Danaus plexippus, Lerodea eufala, Pholisora catullus, Brephidium exilis, Insisalia irus, Philotes sonorensis, Strymon melinus, Alabama argillacea, Helicoverpa zea, Mocis latipes, Ostrinia nubilalis, Spodoptera exigua, Spodoptera frugiperda, Trichoplusia ni, Agrullis vanillae, Brasilarchia archippus, Euptoieta claudia, Vanessa sp, Papilio cresphontes, Papilio palamedes, Colias sp., Eurema daira, Pieris rapae, Acrobasis vaccinii, Amyelosis transitella, Cactoblastis cactorum, Diaphania hyalinata, Diatraea grandiosella, D. lineolata, D. muellerella, Eoreeuma loftini, Hyles lineata, Manduca sexta, Rhopobota naevana, Rhyacionia frustana*. Los hospederos de Lepidóptera fueron encontrados sobre cultivos de algodón, amaranto, alfalfa, almendra, arándano, brócoli, bermuda,

caña de azúcar, calabaza, datura, girasol, lima, malva, maíz, mariguana, papa, pasiflora, pasto, repollo, sauce, sorgo y tomate (Pinto, 1998).

Variabilidad Genética

Los individuos de una misma especie o población no son idénticos. Cuando nos referimos a los cambios que se originan dentro del genoma o material hereditario, le llamamos variabilidad genética. La población presenta varios alelos para el mismo gen que codifica una característica determinada, ésta puede ser cuantificada, en base a su tamaño, fecundidad, longevidad, etc. Determinar, medir y mejorar la variabilidad genética del parasitoide que se reproduce en masa y que se va a liberar, es una condición de éxito en Control Biológico (Chassain & Boulétreau, 1991; Hopper *et al.*, 1993).

Desarrollo de líneas puras

Una línea pura es un grupo de individuos que son homocigotos para gran parte de sus características y éstas se mantienen constantes a través de las generaciones. De una población de campo se pueden obtener líneas puras mediante la autofecundación hermanada (Figura 1).

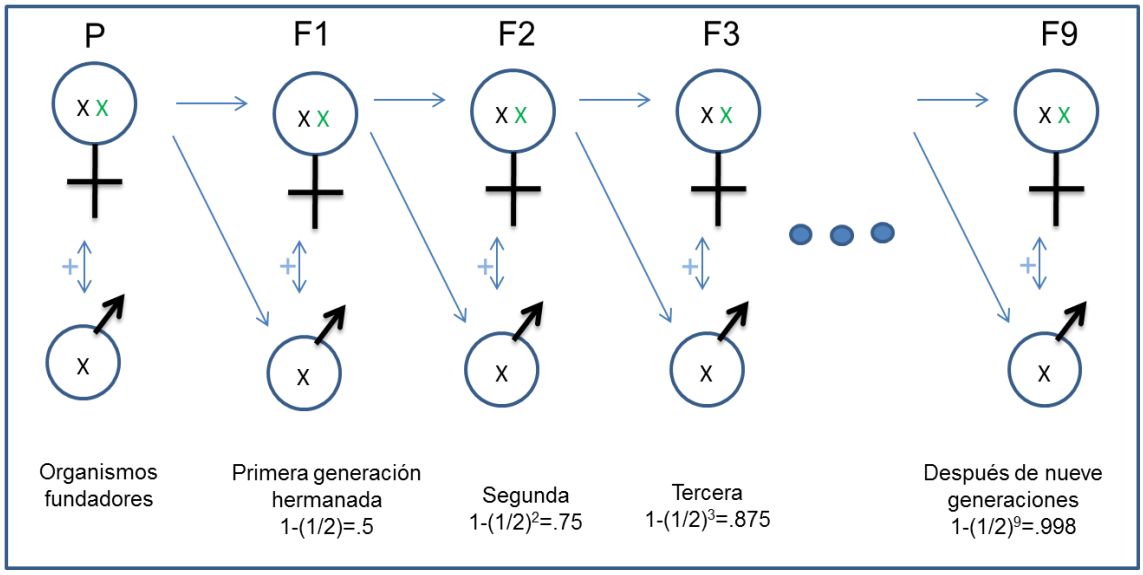

Figura 1 Desarrollo de líneas puras mediante la reproducción hermanada. En un sistema haplodiploide, la hembra (diploide), tiene dos juegos de cromosomas y el macho (haploide), tiene la mitad del número de cromosomas que la hembra.

Mediciones de la variabilidad genética

Para medir la variabilidad genética es necesario identificar características o rasgos del individuo que se transmiten a la descendencia bajo condiciones controladas.

Características o rasgos del individuo

Hopper *et al.* (1993) resume numerosas investigaciones con agentes de control biológico a través de la medición y cuantificación de características o rasgos del individuo que determinan variabilidad genética entre poblaciones, dependiendo del tipo de hospedero, fecundidad, resistencia a insecticidas, mortalidad, tasa sexual y tolerancia a la temperatura. Así mismo, dentro de la población se han desarrollado estudios que predicen variabilidad a través de la

medición del tiempo de desarrollo, fecundidad, longevidad, tasa sexual, preferencia y tolerancia a la temperatura.

Para determinar y comparar el desempeño de líneas puras y poblaciones y elegir aquella con mejor desempeño, se han utilizado distintos métodos. Uno de ellos son las tablas de vida de edad específica, que son consideradas como una herramienta para estimar parámetros de crecimiento, desarrollo y reproducción, al incluir en su análisis sobrevivencia, tiempo y proporción sexual (Lotka, 1907; Lewis, 1942; Leslie, 1945; Southwood, 1978; Bellows Jr *et al.*, 1992; Maia *et al.*, 2000; Badii & Castillo, 2009). Las tablas de vida han sido utilizadas anteriormente para determinar el desempeño entre poblaciones y especies de *Trichogramma* (Pak & Oatman, 1982; Pratissoli & Parra, 2000; Haile *et al.*, 2002; Pratissoli *et al.*, 2004; Samara *et al.*, 2008; Iranipour *et al.*, 2009). Otros métodos relacionados con la evaluación de la calidad y desempeño en parasitoides son aquellos que usan cálculos de fecundidad y proporción sexual (Waage & Ming, 1984; Cerutti & Bigler, 1995; Kazmer & Luck, 1995; Sorati *et al.*, 1996; Antolin, 1999).

Parámetros poblacionales

Los parámetros poblacionales medidos en laboratorio son una abstracción de lo que sucede en la naturaleza sin la existencia de factores ambientales o biológicos, por lo que nos proporcionan una idea de la capacidad máxima de una especie para multiplicarse y son estimados a partir de tablas de vida desarrollados en laboratorio (Southwood & Henderson, 2009). Estos se calculan con base en la sobrevivencia, parasitismo y proporción sexual

observada en cada individuo (hembras) en una unidad de tiempo. Existen dos tipos de tablas de vida: Las tablas en base a la esperanza de vida (no desarrolladas en la presente investigación) y las tablas de vida basadas en la fertilidad y tasas reproductivas (Southwood & Henderson, 2009).

Tablas de vida basadas en la fertilidad y tasas reproductivas

Una tabla basada en la fertilidad se construye a partir de la construcción de las columnas: *x*, intervalo de tiempo (días, semanas, años, etc); *lx,* el número de hembras vivas en un intervalo de tiempo (como una fracción iniciando la población en 1); *mx,* el número de hijas por cada hembra, en cada intervalo de tiempo; y *lx·mx,* la multiplicación de *lx* y *mx*, para obtener el total de hembras nacidas en cada intervalo de tiempo.

Con estas columnas se estiman los siguientes parámetros poblacionales: tasa neta de reproducción ($Ro = \sum lx \cdot mx$), el número de hembras nacidas por cada madre, en cada generación; tiempo generacional ($T = \sum mx \cdot lx \cdot x / \sum mx \cdot lx$) intervalo de tiempo medio, entre el nacimiento de los individuos de una generación y la de la próxima generación; tasa finita de incremento ($\lambda = e^{rm}$) número de individuos que se agrega a la población por individuo y por tiempo; tasa intrínseca de crecimiento, rm ($\sum e^{-rm^x}\ lx \cdot mx = 1$) potencial de crecimiento de una población (Maia *et al.*, 2000).

Fecundidad

La fecundidad tradicionalmente conocida como la capacidad de un individuo o población de reproducirse, ha sido frecuentemente usada *e*n

laboratorios de reproducción masiva por algunos investigadores en *Trichogramma* y cuantificada junto a algunos otros parámetros, para determinar la calidad de los enemigos naturales (Waage & Ming, 1984; Cerutti & Bigler, 1995; Dutton *et al.*, 1996; Kuhlmann U. & Mills N. J., 1999; Pratissoli & Parra, 2000; Pratissoli *et al.*, 2004; Doyon & Boivin, 2005). Un cambio de color en los huevecillos parasitados (de café a negro) ha sido tradicionalmente usado como señal de parasitismo. El conteo directo de huevecillos parasitados facilita la comparación de distintas poblaciones mediante el análisis estadístico de sus varianzas y la comparación de sus medias.

Proporción sexual

Lo más deseable en una producción de parasitoides es obtener un número mayor de hembras, ya que estas influyen directamente en la reducción del hospedero o plaga. La proporción sexual es el número de machos nacidos por hembra entre el número de la progenie total (Wilson & Hardy, 2002). En toda población biológicamente normal y no expuesta a sucesos extraordinarios, ambos sexos representan, prácticamente, la misma proporción (50%). Esta distribución igualitaria se observa sobre todo en poblaciones numerosas. En *Trichogramma* se observa una producción con dominancia de hembras en los primeros días para luego ir cambiando esta relación paulativamente a un mayor número de machos (Kuhlmann U. & Mills N. J., 1999; Guzmán-Larralde *et al.*, 2013). La causa en la baja de individuos en el tiempo y el cambio en la proporción de sexos ha sido explicado por el agotamiento de esperma

almacenado en la hembra joven o producto de la edad en las avispas (Chassain & Boulétreau, 1991).

Tamaño

El tamaño ha sido utilizado en la identificación de especies así como en la relación de este parámetro con distintas características. Ha sido correlacionado con el hospedero (Bai *et al.*, 1992), nutrición (Greenberg *et al.*, 2000) , fecundidad (Smith, 1996; Ellers & Jervis, 2003) y recientemente con la variabilidad genética (Sorati *et al.*, 1996). Es un atributo útil y fácil de medir en los individuos. Tradicionalmente se han utilizado mediciones de distintas partes del cuerpo en Trichogramma como cabeza, tórax, tibia, longitud del ala, entre otras (García-González *et al.*, 2011).

Artículo Científico I

Genetic variation and the performance of a mass-reared parasitoid, *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), in laboratory trials

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Genetic variation and the performance of a mass-reared parasitoid, *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), in laboratory trials

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Abstract

During mass rearing, adaptation of biological control agents to the rearing environment is a potential problem. Using the parasitoid wasp *Trichogramma pretiosum*, the performance of 26 highly inbred lines, five composite 'populations' (created from the inbred lines) and one insectary-reared population was compared using fertility life tables. Of the composite populations, three were created with maximal and identical genetic variation as a mixture of all 26 inbred lines, but these were then reared for a different number of generations (2, 6 or 17) before their performance was measured. The remaining two composite populations were created based on the performance of the individual inbred lines: one was a combination of two inbred lines with a high intrinsic rate of natural increase (rm), 'high rm'; and the other was a combination of two lines with a 'low rm'. High and low rm populations were reared for two generations prior to testing. Parameters measured were fertility, longevity and sex ratio. We found no difference between the maximally variable population reared for two generations and the 'high rm' population (rm = 0.285 and 0.282, respectively). 'Low rm' was the population with the lower performance (rm = 0.255). Genetically variable population reared for two generations for 48 h produced significantly more offspring than the populations reared for 6 and 17 generations. Hybrid population derived from the high-rm lines did significantly better than that derived from the low-rm lines. Low-performance populations become more male based than high performance at 48 h. The potential benefits to improve population's performance using inbred lines for mass rearing are discussed.

Introduction

In classical and augmentative (inoculative or inundative) biological control programmes, genetic variation within a parasitoid release promotes adaptability to the environment (Chassain and Boulétreau 1991; Hopper et al. 1993; Wajnberg 2004). However, maintaining genetic variability during the process of mass rearing is difficult. Loss of variation may occur at the start of the captive rearing, because some individuals better adapted to the artificial rearing conditions have advantages over others, therefore leading to elimination of some alleles and domestication (Bartlett 1984; Allendorf 1986; Nunney et al. 2002). In addition, emphasis on increasing production quantity may also lead to a decrease in quality of the population (Nunney 2003), and inbreeding and genetic adaptation during prolonged mass rearing may lead to a suboptimal performance in the field (Woodworth et al. 2002; Frankham 2005).

One approach to mitigate loss of genetic variation, and therefore quality, has been to simply find an inbred line that combines an optimal performance both during the mass rearing and in the field (Roush and Hopper 1995; Margan et al. 1998; Kalyebi et al. 2005; Nunney 2006). As such inbred lines lack genetic variation, no genetic change can take place, except through mutations. That such completely homozygous lines can remain useful is illustrated by *Encarsia formosa*, a 100% homozygous, thelytokous species, used practically continuously since the 1930s for the successful control of the greenhouse whitefly in Europe (van Lenteren et al. 1997).

However, an alternative approach that may be used for augmentative releases is dividing the founding population into subpopulations. Each of these subpopulations is expected to lose a different set of alleles during their establishment and subsequent prolonged rearing, but the original variation can be largely restored by mixing the subpopulations shortly before they are released in the field (Hopper et al. 1993; Margan et al. 1998; David et al. 2004; Nunney 2006). To maintain and restore 95% of the common alleles, present in the original field population, some authors suggest hybridizing at least 25 inbred lines, when working with haplodiploid insect parasitoids (Roush and Hopper 1995; Nunney 2006).

Trichogramma pretiosum is a minute wasp that is frequently used in biological control programmes against lepidopteran pests. Its ease of rearing and handling make it an ideal candidate organism for investigating genetic processes affecting the performance of mass-reared biological control agents.

To determine the performance of populations, fertility life tables are considered the best tool to estimate parameters of growth, development and reproduction (Lotka 1907; Lewis 1942; Leslie 1945; Southwood 1978; Bellows et al. 1992; Maia et al. 2000; Badii and Castillo 2009). Four parameters associated with a fertility life table have been used in laboratory studies to differentiate reproductive potential at various temperatures and hosts in many studies on *Trichogramma* species (Pak and Oatman 1982; Pratissoli and Parra 2000; Haile et al. 2002; Pratissoli et al. 2004a,b; Samara et al. 2008; Iranipour et al. 2009). The intrinsic rate natural of increase (r_m) developed by Lotka (1945) is a very useful parameter describing the biotic potential population growth of insect populations under laboratory conditions because it combines fecundity, sex ratio and survival rate (Birch 1948).

Alternatively, parasitism and sex ratio (together and separately) have also been used to understand the reproductive strategy of *Trichogramma* populations (Waage and Ming 1984; Hoffmann et al. 2001). Chassain and Boulétreau 1991 compared the variability in sex ratio and progeny allocation among *Trichogramma brassicae* strains collected from three different locations over two successive generations, finding differences between the small populations, genetic intraspecific variability, and showing that sex ratio and progeny allocation were heritable and relatively stable.

The present work aimed to assess the use of *T. pretiosum* inbred lines to understand parasitism performance under laboratory conditions. Differences between generation time and variability of inbred lines were determined using fertility life tables and the calculation of the 'intrinsic rate of natural increase' (r_m) as a predictor of performance. The ultimate purpose of this study is to provide decision tools for the mass rearing of *Trichogramma* and evidence of the benefits of isofemale line's methodology to avoid domestication while at the same time maintaining genetic variability.

Materials and Methods

Source of experimental inbred lines

Twenty-six inbred *T. pretiosum* lines were initiated from parasitized *Manduca sexta* eggs collected from tomato fields at the University of California's field station in Irvine, California, in the summer of 2008. Single-mated females, each emerging from a different field-collected host egg together with presumably siblings, were used to initiate the inbred lines; each line was inbred by brother–sister mating for nine generations. After that, inbred lines were reared in populations of several hundreds of individuals. All lines were maintained on UV-irradiated *Ephestia kuehniella* eggs in a temperature cabinet at 25°C, 16/8-h light/dark period and 30% relative humidity.

Performance of individual inbred lines

Twenty-five mated females 12–24 h old were collected and placed in individual 12 × 75 mm glass vials, each containing an egg card with approximately 150 host eggs and a small streak of honey (food source). Vials were checked daily, and egg cards were renewed every 2 days until the female died. Parasitized egg cards were maintained in individual vials, and following emergence, offspring were sexed and counted.

Southwood (1978) and Maia et al. (2000) methodology was used to calculate population parameters and fecundity life tables of the 26 inbred lines. The *mx*

is the age-specific fertility, or reproductive rate, and was obtained by dividing the number of daughters produced during every 2-day period by the number of mothers; lx is the number of surviving females living at the end of every 2-day period. The net reproductive rate ($Ro = \sum lxmx$) is the number of newborn females per mother per generation. The mean generation time ($T = \sum mxlx.x / \sum mxlx$) is the mean time span between the birth of individuals of a generation and that of the next generation. The finite rate of increase ($\lambda = e^{rm}$) is the number of offspring born per female during a finite time. The intrinsic rate of natural increase, rm, calculated using the formula ($\sum e^{-rm*x} lxmx = 1$), represents the growth potential of a population under given climatic and food conditions. The number of offspring produced in the first 48 h, lifetime fecundity and the sex ratios [proportion male (Wilson and Hardy 2002)] of the offspring were recorded. The relative costs were calculated using the formula given below:

$$\text{relative cost} = \frac{\text{offspring production}}{\text{female}}$$

Performance of populations

The 26 inbred lines were imported to Mexico from UC Riverside and used to create five experimental populations. The first three populations were created equal, each consisting of a combination of all 26 inbred lines, thus maximizing initial genetic variation. However, the three populations were subject to different times of rearing (2, 6 or 17 generations) before measuring their performance. These three populations were constructed separately at a different time to synchronize all populations at the end. Two further populations were created using a combination of only two inbred lines, selection based on their rm. The first population called 'high rm' was constructed using one inbred line with high rm (LP43) and one with an intermediate rm (LP35); the second population called 'low rm' was constructed using one inbred line with a low rm (LP9) and one with an intermediate rm (LP33). The high rm and low rm populations were tested for two generations after they were initiated. A final population was added to the experiment from the 'mass rearing facility' in Saltillo, Coahuila, Mexico. The initial genetic make-up of this population was unknown, but it had been maintained in the rearing facility for more than 2 years.

Experiments to collect the data for the life tables of these six populations were performed simultaneously. The population parameter determination and fecundity/sex ratio estimates were the same as for the inbred lines, except that we increased the sample size to 50 females per population and the host eggs used were *Sitotroga cerealella* instead of *E. kuehniella* (not available in Mexico).

Data analysis

Data were analysed using one-way ANOVA's multiple comparisons using Minitab for Windows (version 16; Minitab Inc., State College, PA). Lines and populations with significant differences were tested *post hoc* using the Tukey's exact test and Bonferroni correction. Sex ratio data were arcsine-square-root-transformed prior to analysis.

Results

Inbred lines

Population parameters of the 26 inbred lines of *T. pretiosum* are shown in table 1. Ro ranged from 5.75 (LP9) to 54.13 (LP46), and λ was >1 for all lines, predicting that all of them would increase in population size under laboratory conditions. The growth potential of each line, estimated as rm, ranged from 0.13 (LP9) to 0.29 (LP43), and mean generation time (T) ranged from 13.04 (LP47) to 15.37 (LP23) days, with a mean of 14.35 days.

Across the 26 inbred lines, significant differences existed in both 48-h ($F_{25,624} = 9.10$; $P < 0.001$) and lifetime fecundity ($F_{25,624} = 7.82$; $P < 0.001$). For 48-h fecundity, *post hoc* tests revealed two distinctive groups with particularly low (lines 2, 9 and 53) and high fecundity (lines 29, 35, 43 and 47) (table 2). For lifetime fecundity, *post hoc* tests also revealed two distinctive groups: one group with particularly low fecundity (lines 2, 9 and 53) and one group with particularly high fecundity (lines 27 and 29) (table 2). Significant differences existed between the sex ratios of the inbred lines ($F_{25,498} = 4.05$; $P < 0.001$) ranging from 64% male (line 29) to only 18% male (line 46) (table 3).

There was a positive correlation $R^2 = 0.6522$ between 48-h fecundity and intrinsic rate of natural increase in 26 isofemale lines (fig. 1). Although the lines with the highest 48-h and lifetime fecundity differed somewhat, there was a positive correlation $R^2 = 0.671$ between 48-h female fecundity and life time female fecundity in 26 isofemale lines (fig. 2).

All inbred lines produced female-biased sex ratios over the first 2-day period. The fraction of lines that

Table 1 Fertility population parameters and lifetime and 48-h sex ratio values (% males) of inbred *Trichogramma pretiosum* lines reared in *Ephestia kuehniella* eggs

Isofemale line	Fertility population parameters				Sex ratio	
	Ro	*T*	λ	*rm*	48 h	Life time
43	50.26	14.18	1.34	0.29	0.10	0.35
39	51.98	14.63	1.33	0.28	0.11	0.27
46	54.13	14.90	1.33	0.28	0.13	0.18
28	54.11	15.12	1.32	0.28	0.16	0.27
29	50.15	14.88	1.32	0.28	0.19	0.64
6	45.86	14.80	1.31	0.27	0.20	0.35
27	44.81	14.88	1.31	0.27	0.16	0.38
51	40.60	14.75	1.30	0.27	0.21	0.33
35	31.60	13.17	1.30	0.27	0.22	0.43
40	35.08	14.05	1.30	0.27	0.12	0.36
23	44.10	15.37	1.29	0.26	0.20	0.37
42	33.30	13.86	1.29	0.26	0.17	0.40
3	31.48	13.98	1.29	0.26	0.13	0.22
47	26.19	13.04	1.29	0.26	0.30	0.52
22	33.72	14.73	1.29	0.26	0.24	0.49
12	32.46	14.42	1.28	0.25	0.24	0.37
52	31.34	14.98	1.27	0.24	0.19	0.45
14	27.75	14.60	1.27	0.24	0.30	0.37
37	26.60	14.17	1.27	0.24	0.25	0.38
26	27.06	15.04	1.26	0.24	0.25	0.32
33	28.52	15.19	1.26	0.23	0.22	0.34
1	24.30	14.38	1.26	0.23	0.30	0.46
53	21.78	14.53	1.25	0.22	0.38	0.44
38	15.14	13.51	1.23	0.21	0.31	0.47
2	7.85	14.66	1.16	0.15	0.25	0.52
9	5.75	13.33	1.14	0.13	0.41	0.50

Ro, net reproductive rate (newborn females per mother per generation), *T*, mean generation time, λ, finite rate of increase, *rm*, intrinsic rate of increase (growth potential of a population)

Temperature 25°C, 16/8-h light/dark and 30% relative humidity. Twenty-five females per replicate.

produced female-biased offspring declined over time (fig. 3). The relative cost of a female (expressed as the total number of offspring produced during the 2-day period divided by the number of females produced during that time) increases over time (fig. 4). Here we assume that all host eggs are parasitized, which rarely will be the case.

Populations

The population parameters of six populations are shown in table 4. *rm* ranged between a high value found in 'genetically variable population reared for two generations' (*rm* = 0.285) and a low value for the population 'low *rm*' (*rm* = 0.255). The second highest *rm* value was found in the 'high *rm*' population (*rm* = 0.282), a population obtained by mixing only two inbred lines, and had a higher value than the 'genetically variable population reared for 6 and 17 generations' (*rm* = 0.275 and *rm* = 0.276, respectively) and also over 'mass rearing population'. Ro was 45.44 for the 'genetically variable population reared for two generations' and 33.46 for the 'low *rm*' population. The shortest *T* corresponded to the 'high *rm*' population (*T* = 13.56) and the longest to the group from the 'low *rm*' population (*T* = 14.43).

Across the six populations, significant differences existed in both 48-h ($F_{5,294} = 6.12$; $P < 0.001$) and lifetime fecundity ($F_{5,294} = 3.10$; $P = 0.010$). For 48-h fecundity, *post hoc* tests revealed two distinctive groups, one group with particularly high fecundity, consisting of the 'high *rm*' and 'genetically variable population' reared for two generations, and one group with particularly low fecundity, consisting of the 'genetically variable population' reared for 6 and 17 generations, 'mass rearing' and 'low *rm*', see table 5. For the lifetime fecundity, *post hoc* test also revealed two distinctive groups, and most of the population means differ significantly from the 'low *rm*' population, but there are no significant differences among the 'genetically variable population' reared for 2, 6 and 17 generations, 'high *rm*' and 'mass rearing' populations, see table 5.

All six populations had a female-biased sex ratio over their lifetime, and mean sex ratios did not differ significantly among the populations ($F_{5,269} = 0.30$; $P = 0.912$). For 48-h sex ratio, significant differences were found ($F_{5,273} = 20.08$; $P < 0.001$). *Post hoc* tests indicate three groups, one particularly high sex ratio group consisting of the 'mass rearing' and 'low *rm*', followed by 'genetically variable' population reared for 2, 6 and 17 generations and the low sex ratio group 'high *rm*', see table 6.

Discussion

Fertility life tables represent an important biological tool to evaluate the potential population growth of different lines. Several studies have used different *T. pretiosum* populations, host and temperature to determine performance (Pak and Oatman 1982; Pratissoli and Parra 2000; Pratissoli et al. 2004b). Genetic variation is a further important component affecting population performance. In the present study, 26 inbred *T. pretiosum* lines were derived from a single field population. Inbreeding within the sibmated isofemale lines was performed to expose the genetic variability present in the original field population. We determined the performance of each inbred

Table 2 Mean (±SE) of 48-h and lifetime fecundity of inbred *Trichogramma pretiosum* lines reared on *Ephestia kuehniella* eggs

	48-h fecundity									Lifetime fecundity								
Line	Mean	Group								Line	Mean	Group						
43	28.04 ± 2.4	A								29	107.52 ± 10.2	A						
47	26.36 ± 2.9	A	B							27	92.88 ± 13.1	A	B					
35	26.12 ± 1.1	A	B							43	87.28 ± 8.2	A	B	C				
29	25.60 ± 1.6	A	B							28	85.00 ± 8.0	A	B	C				
3	23.56 ± 1.9	A	B	C						23	84.60 ± 10.9	A	B	C				
28	23.40 ± 1.7	A	B	C						6	82.96 ± 9.0	A	B	C				
6	22.44 ± 1.6	A	B	C						22	79.16 ± 11.2	A	B	C	D			
51	21.96 ± 2.4	A	B	C						46	72.96 ± 9.0	A	B	C	D			
22	20.76 ± 2.9	A	B	C	D					51	69.20 ± 8.5	A	B	C	D	E		
40	20.56 ± 2.4	A	B	C	D					52	67.92 ± 11.3	A	B	C	D	E		
42	20.52 ± 2.1	A	B	C	D					42	67.60 ± 7.9	A	B	C	D	E		
46	20.28 ± 1.9	A	B	C	D					39	67.20 ± 7.5	A	B	C	D	E		
27	20.04 ± 2.5	A	B	C	D					40	66.12 ± 9.1	A	B	C	D	E	F	
12	18.84 ± 1.4	A	B	C	D	E				35	62.92 ± 4.2	A	B	C	D	E	F	G
23	18.40 ± 2.3	A	B	C	D	E	F			47	62.76 ± 6.9	A	B	C	D	E	F	G
39	17.48 ± 1.7	A	B	C	D	E	F			12	61.68 ± 6.5	A	B	C	D	E	F	G
14	16.80 ± 2.1	A	B	C	D	E	F			1	50.40 ± 6.2		B	C	D	E	F	G
26	15.84 ± 2.6		B	C	D	E	F	G		33	48.08 ± 9.2		B	C	D	E	F	G
37	15.52 ± 1.3		B	C	D	E	F	G		14	47.84 ± 7.8		B	C	D	E	F	G
1	13.84 ± 1.8			C	D	E	F	G		26	45.72 ± 7.5			C	D	E	F	G
52	13.32 ± 2.0			C	D	E	F	G		37	44.52 ± 4.2			C	D	E	F	G
38	13.04 ± 2.3			C	D	E	F	G		3	43.32 ± 4.2			C	D	E	F	G
33	9.76 ± 2.4				D	E	F	G		38	39.52 ± 6.4				D	E	F	G
9	8.08 ± 1.6					E	F	G		2	26.60 ± 6.7					E	F	G
2	7.04 ± 1.7						F	G		53	20.16 ± 5.2						F	G
53	5.08 ± 1.4							G		9	17.88 ± 5.2							G

SE, standard errors.

Means followed by the same letter were not significantly different, Tukey's exact test ($P < 0.001$). Temperature 25°C, 16/8-h light/dark and 30% relative humidity. Twenty-five females per replicate.

line using two measures, rm and fecundity, revealing large range of values. Based on rm, the highest-performing inbred line showed a two times higher intrinsic rate of natural increase (rm) and nine times higher net reproductive rate (Ro), than the lowest-performing line. Similarly, based on fecundity, the highest-performing line also had a six times higher lifetime fecundity and five times higher 48-h fecundity than the lowest-performing line. Similar observations have been previously reported; inbreeding exposes the heritable genetic variation, and sibling inbred line reared under same conditions differ in laboratory performance (Cerutti and Bigler 1995; Sorati et al. 1996; Thomson and Hoffmann 2002).

Our data were similar to that reported by Pratissoli et al. (2004a) with the same host used in our experiments, *E. kuehniella*. They found a Ro = 31.53, lower than our genetically variable population, but a shorter generation time ($T = 10.86$), $\lambda = 1.37$ and a higher rm ($rm = 0.32$).

Pak and Oatman (1982) studied *T. pretiosum* laboratory population, reared at least for 4 years, and found mean lifetime offspring production of 51.3 at 25°C in *Trichoplusia ni*, lower than what we found in our experiment (59.78–84.70) using *E. kuehniella*. This is unexpected because *Trichogramma* females emerging from *T. ni*. eggs are generally large and more fecund than the smaller females emerging from *E. kuehniella* (Melton 1983; Hohmann et al. 1988; Bai et al. 1992). One possible explanation for this difference could be the longer period under which the population used in the 1982 study was reared in the laboratory.

The correlation between rm and fecundity was positive ($R^2 = 0.6522$; $P < 0.001$). Both parameters jointly are good decision tools. rm provides a rate to determine which line or population produces a greater number of daughters through time, and fecundity is statistically useful to determine significant differences between offspring production among populations.

Table 3 Lifetime offspring sex ratio and standard error of inbred *Trichogramma pretiosum* lines reared on *Ephestia kuehniella* eggs

Line	N	Sex ratio (% male)	SE	Group			
29	21	0.64	0.06	A			
2	12	0.53	0.05	A	B	C	
47	21	0.52	0.05	A	B		
9	18	0.50	0.05	A	B	C	
22	18	0.49	0.05	A	B	C	
38	16	0.47	0.05	A	B	C	D
1	22	0.46	0.06	A	B	C	
52	17	0.45	0.04	A	B	C	D
53	12	0.44	0.06	A	B	C	D
35	25	0.43	0.06	A	B	C	D
42	24	0.40	0.05	A	B	C	D
27	19	0.38	0.05	A	B	C	D
37	28	0.38	0.03	A	B	C	D
14	19	0.37	0.06	A	B	C	D
28	21	0.37	0.05	A	B	C	D
12	24	0.37	0.03	A	B	C	D
40	19	0.36	0.04	A	B	C	D
6	28	0.35	0.06		B	C	D
43	22	0.35	0.04		B	C	D
33	17	0.34	0.02	A	B	C	D
51	20	0.33	0.03		B	C	D
26	18	0.32	0.04		B	C	D
39	28	0.27	0.04		B	C	D
28	28	0.27	0.03		B	C	D
3	24	0.22	0.04			C	D
46	28	0.18	0.02				D

Means followed by the same letter were not significantly different, Tukey's exact test ($P < 0.001$). Temperature 25°C, 16:8 h light:dark and 30% relative humidity.

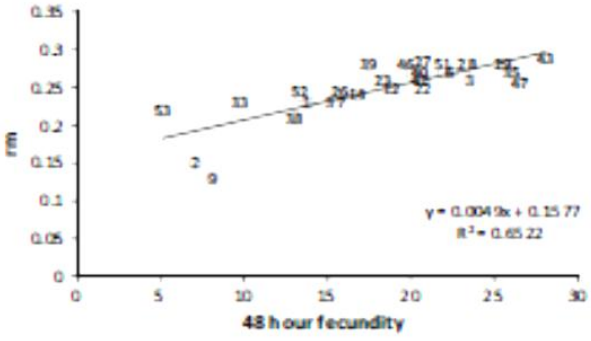

Fig. 1 Relationship between 48 h fecundity and intrinsic rate of increase of 26 inbred lines of *Trichogramma pretiosum* reared on *Ephestia kuehniella* eggs.

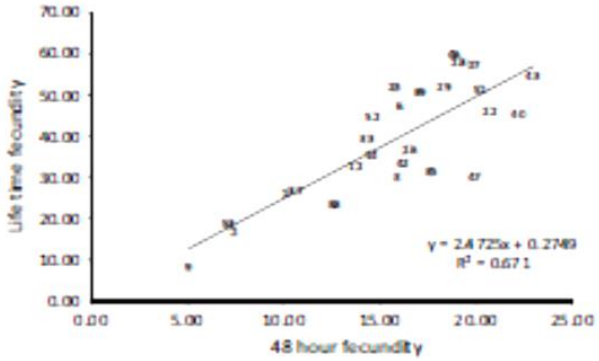

Fig. 2 Relationship between lifetime and 48 h female offspring production in 26 inbred lines of *Trichogramma pretiosum*, reared on *Ephestia kuehniella* eggs.

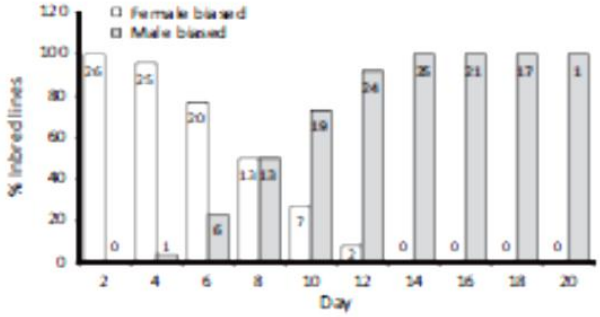

Fig. 3 Percentage and number of inbred lines of *Trichogramma pretiosum* that produced female or male biased offspring sex ratios, for each 2 day period of oviposition.

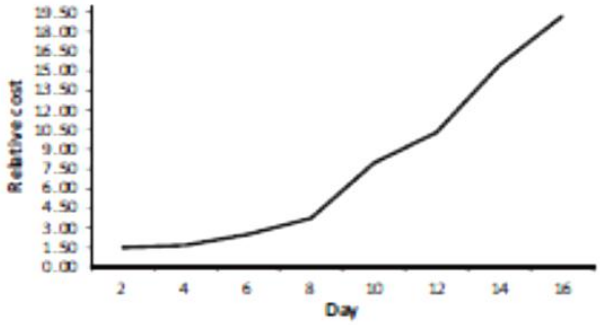

Fig. 4 Relative cost of producing a female *Trichogramma pretiosum* during each 2 day period expressed as the total number of offspring produced divided by the number of females produced during that period.

One of the expectations of prolonged mass rearing is that a genetically variable population should, over time, adapt to the mass rearing conditions. This should result in an increasing performance. Our results indicate, contrary to expectations that no significant changes are detected in life time offspring

Table 4 Population parameters, lifetime and 48-h sex ratio values, of six *Trichogramma pretiosum* populations reared in *Sitotroga cerealella* eggs

Parameters	Genetically variable populations			'High rm' population	'Low rm' population	Mass rearing population
	2 generations	6 generations	17 generations			
rm	0.285	0.275	0.276	0.282	0.255	0.262
Ro	45.44	44.4	45.26	40.97	33.46	35.45
T	13.9	14.35	14.41	13.56	14.43	14.29
λ	1.33	1.32	1.31	1.33	1.29	1.3
48-h sex ratio	0.17	0.18	0.21	0.08	0.33	0.40
Lifetime sex ratio	0.38	0.35	0.36	0.36	0.36	0.42

rm, intrinsic rate of increase (growth potential of a population), Ro, net reproductive rate (newborn females per mother per generation), *T*, mean generation time, λ, finite rate of increase.

Temperature 25°C, 16:8-h light:dark and 30% relative humidity. Fifty females per replicate.

Table 5 Mean (±SE) 48-h and lifetime fecundity of six *Trichogramma pretiosum* populations reared in *Sitotroga cerealella* eggs

Populations	48-h fecundity			Lifetime fecundity		
	Mean	Group		Mean	Group	
'High rm'	20.78 ± 3.07	A		79.60 ± 13.12	A	
2 generations	19.70 ± 2.76	A		84.70 ± 15.34	A	
17 generations	16.06 ± 2.44		B	84.16 ± 17.67	A	
6 generations	15.34 ± 2.76		B	82.02 ± 17.04	A	
'mass rearing'	15.04 ± 4.20		B	66.38 ± 20.94	A	B
'Low rm'	13.98 ± 3.35		B	59.78 ± 15.63		B

SE, standard errors.

Means followed by the same letter were not significantly different, Tukey's exact test (48 h $P < 0.001$) (lifetime $P = 0.010$). Temperature 25°C, 16/8 h light/dark and 30% relative humidity. Fifty females per replicate.

Table 6 Mean 48-h sex ratio and standard errors of six *Trichogramma pretiosum* populations reared in *Sitotroga cerealella* eggs

Populations	N	Mean	SE	Group		
'mass rearing'	41	0.40	0.03	A		
'Low rm'	46	0.33	0.03	A		
17 generations	48	0.21	0.01		B	
6 generations	46	0.18	0.01		B	
2 generations	40	0.17	0.01		B	
'High rm'	40	0.08	0.02			C

Means followed by the same letter were not significantly different, Tukey's exact test ($P < 0.001$). Temperature 25°C, 16/8 h light/dark and 30% relative humidity. Fifty females per replicate.

production between the genetically variable populations reared for 2, 6 and 17 generations. This is consistent with Prezotti et al. (2004), who found no evidence that *T. pretiosum* reared for 25 generations, showed significant alterations in biological features. When we look for such effects in 48-h fecundity, we found that the genetically variable population reared for two generations produced significantly more offspring than the populations reared for 6 and 17 generations. One would expect that the 48-h fecundity is under selection more so than life time fecundity under mass rearing conditions because new host eggs are supplied as soon as the first wasps emerge and most of the host eggs will be parasitized within 48 h.

An alternative explanation for the initially higher rm, fecundity and female production for the genetically variable population reared for two generations and 'high rm' populations could be that initially, a high level of heterozygosity will be present in the population that may result in a higher fitness, but over time, this is lost by drift and inbreeding. However, the size at which these populations were kept would preclude a rapid loss of genetic variation.

The two populations constructed by mixing two high-rm inbred isofemale lines and two lower rm inbred lines performed as expected, and the hybrid population derived from the high-rm lines did significantly better than that derived from the low-rm lines. Our results show also that mixing these two high-rm lines was comparable with a population formed by mixing 26 inbred lines. A simpler solution may be to select the best-performing inbred line; unfortunately, we never tested that line against the mixed lines using the same host eggs, and consequently, we cannot compare them. Clearly, such effects would need to be studied in follow-up experiments.

In this study, female-biased offspring production occurs during the first few days of oviposition, but decreases with time. On average, 77–50% of all lines produce a majority of females until days 6–8; this is consistent with Kuhlmann and Mills (1999) who found female-biased offspring production in *T. pretio-*

sion for the first 5–6 days. The change in sex ratio over time has been explained by the depletion of sperm or age as a general cause of lower fertilization rates (Chassain and Boulétreau 1991). As the goal of mass rearing is to produce females for biological control, using female parents for more than 6 days in a mass rearing may be counterproductive because from that time, the relative cost of production of female wasps will be increasing (see fig. 4), and she will be using the valuable host eggs in an inefficient way by producing more sons than daughters. The most efficient way of producing females for release is to only allow a mother to produce offspring for 4 days, because under those circumstances, the mean number of hosts used to produce a female offspring will be the lowest.

If our fertility life table results are at all an indication of the potential range of performances that can be expected when wasps are released in classical biological control projects, it is clear that failures can easily occur if only a few individuals are used to establish populations that will be used for field releases. If inbred populations of the potential biocontrol agent established in mass rearing have a low field performance, the biocontrol agent may be deemed unsuitable for control of the pest. Yet, if high-performance inbred lines or genetically variable populations had been released, biocontrol would have succeeded.

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Artículo Científico II

Genetic variation, size and fecundity effect in *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) inbreed lines

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BioControl

Genetic variation in size-fecundity relationships in the parasitoid wasp Trichogramma pretiosum

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Abstract:	We determined why in some studies a strong correlation is found between size of females of the egg parasitoid Trichogramma (Hymenoptera: Trichogrammatidae) and their lifetime fecundity, while in others no such relationship is found. We compared the relationship between size and lifetime fecundity in several different populations of T. pretiosum Riley: 6 populations each consisting of a different highly inbred line, a population consisting of a mixture of the 6 inbred lines and a mixture of 26 highly inbred lines. The performance of the highly inbred populations formed a consistent hierarchical ranking in trials performed over 2 years apart, with females of any given size from certain lines, on average being more fecund than females of the same size from a different line. In comparison, the population created as a mix of the highly inbred lines, performed as an "average" population, and the correlation between wasp size and fecundity was lost.
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abstract

Abstract

We determined why in some studies a strong correlation is found between size of females of the egg parasitoid *Trichogramma* (Hymenoptera: Trichogrammatidae) and their lifetime fecundity, while in others no such relationship is found. We compared the relationship between size and lifetime fecundity in several different populations of *T. pretiosum* Riley: 6 populations each consisting of a different highly inbred line, a population consisting of a mixture of the 6 inbred lines and a mixture of 26 highly inbred lines. The performance of the highly inbred populations formed a consistent hierarchical ranking in trials performed over 2 years apart, with females of any given size from certain lines, on average being more fecund than females of the same size from a different line. In comparison, the population created as a mix of the highly inbred lines, performed as an "average" population, and the correlation between wasp size and fecundity was lost.

Genetic variation in size-fecundity relationships in the parasitoid wasp *Trichogramma pretiosum*

Abstract

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Keywords: Hymenoptera, *Trichogramma pretiosum*, isofemale lines, hind tibia length, laboratory performance, mass rearing

INTRODUCTION

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) is an egg parasitoid frequently used in biological control programs against lepidopteran pests, and can be considered a model organism for understanding genetic processes affecting the performance of mass reared biological control agents. Quality control is required in the mass production of natural enemies to assure optimal performance in the field (Bigler 1989; Cerutti and Bigler 1995; van Lenteren, 2003). It has been shown that individual female size has consequences for reproductive success, and hence, for the performance of a mass reared colony (van den Assem et al. 1989). Parasitoid size is an easily measured quality parameter, and independent studies in Encyrtidae and Pteromalidae have shown a positive correlation between size and fecundity (van den Assem et al. 1989; Sagarra et al. 2001).

Many studies show that the size of egg parasitoids is influenced by the size of the host egg in which they develop (Salt 1940; Schmidt and Smith 1987; Hohmann et al. 1988; Bai et al. 1992; Corrigan and Laing 1994; Bjorksten and Hoffmann 1998; Honda and Luck 2001; Rukmowati-Brotodjojo and Walter 2006; Hamed and Nadeem 2012). The number of wasp larva developing in an egg also influences the size of the resulting adult wasps; the higher the number of larvae per egg the smaller the emerging adults (Waage and Ming 1984; Durocher-Granger et al. 2011). The relationship between wasp size and fecundity in *Trichogramma* has been investigated multiple times, but remains controversial due to the influence of factors like species identity (of both parasitoid and host), size of the host egg, nutritional state (of both the ovipositing parasitoid and its host), and the genetic make-up of individual wasps (Smith 1996; Ellers and Jervis 2003). As a result, some experiments have found a weak or no correlation between, size and performance (Pavlik 1993; Sorati et al.

45 1996). In addition, it has been shown that artificial sources of carbohydrates in laboratory
46 experiments also affects fecundity (Bai et al. 1992; Greenberg et al. 2000; Gurr and Nicol
47 2000; Hegazi et al. 2000; Saljoqi and Khajjak 2007).

48 Differences between size and performance may also be related to intraspecific
49 genetic differences or environmentally induced phenotypic plasticity (Pinto et al. 1989).
50 Previous studies were also often performed using genetically variable populations.
51 Therefore, genetic variation for the measured traits could have confounded any
52 performance measures. To avoid such problems, the use of inbred populations may reduce
53 within line genetic variation, although in inbred strains of *T. brassicae* Bezdenko, fitness
54 measures were still not correlated with size (Sorati et al. 1996).

55 Prolonged mass rearing of a population with high initial genetic variability, results
56 in selection for mass rearing conditions, and consequently cause a decrease in performance
57 under field conditions (Sorati et al. 1996; Nunney 2003). Among other things, decreased
58 quality may manifest itself as a reduction in size and/or fecundity. One potential solution to
59 this problem is to rear a population as multiple smaller populations, each created as a single
60 isofemale line. Such highly inbred lines are expected to lose a different set of alleles during
61 their establishment and prolonged rearing, but taken as a whole, they will retain a much
62 larger amount of the variation present in the initial population. Thus, the genetically
63 variable population initially brought into rearing, can be largely reconstituted by mixing the
64 isofemale lines prior to release.

65 Here we investigate the relationship between size and offspring production, utilizing
66 several genetically different, but highly inbred lines, and a genetically variable population
67 that has been mass-reared for differing lengths of time.

68

69 MATERIALS AND METHODS

70 All experiments were done using a temperature cabinet at 25 °C, 16/8 h light/dark
71 and 30% relative humidity. The *T. pretiosum* inbred lines used in this research were derived
72 from parasitized *Manduca sexta* L. (Lepidoptera: Sphingidae) eggs collected in tomato
73 fields at the University of California's field station in Irvine, California, in the summer of
74 2008. Single mated females, each emerging from a different field collected host egg
75 together with presumably siblings, were used to initiate the inbred lines; each line was
76 inbred by brother sister mating for nine generations using *Ephestia kuehniella* Zeller
77 (Lepidoptera: Pyralidae) eggs as hosts. The resulting isofemale lines should have an
78 inbreeding coefficient of at least 86% (Li 1955). After investigating fecundity (over a 48h
79 period following mating) in 26 inbred lines in a previous study (Guzmán-Larralde et al.
80 2013), six inbred lines with contrasting performance were used.

81 To understand differences in the relationship between size and fecundity as a
82 function of genetic variation within lines three studies were performed. First, we compared
83 size, fecundity and generation time in six inbred lines; two with high (lines 29 and 43), two
84 with low fecundity (lines 2 and 53), and two with intermediate fecundity (lines 1 and 9)
85 (see Guzman-Larralde et al. 2013). Two trials were performed (February 2011 and April
86 2013) to investigate temporal variation and any potential change in performance with time

87 in rearing. Second, the performance of these 6 inbred lines was compared with the
88 performance of a population created by mixing all of them. This mixed population was
89 reared for 2 generations before performance measurements were taken. Finally, differences
90 in size and fecundity were sought between a highly genetically variable population (created
91 from 26 inbred lines) after 2 and 40 generations of laboratory rearing.

92 For each inbred line and the populations created by mixing them, 25 mated females,
93 up to 24 h old, were collected and each female placed individually in a glass tube with
94 approximately 400 eggs for oviposition and a drop of honey as a food source. For each of
95 the two populations with high initial genetic variation, 50 mated females were sampled.
96 Only *E. kuehniella* eggs were used to avoid host size influences. After 48 h, all females
97 (mothers) were removed from the tube and measured. Fecundity was assessed as the
98 number of offspring produced over the first 48 h period, where parasitized host eggs are
99 recognized by their color change from clear to dark. Females without offspring were
100 discarded. Female size was estimated as hind tibia length (HTL), determined under an
101 optical microscope at 40X with a calibrated micrometer, using methods described in
102 Hohmann et al (1988) .

103 Statistical analyses

104 All statistics were performed using Minitab version 16 (Minitab Inc., PA, USA). To
105 control for within line/population variation in body size, residual fecundity was calculated
106 by regressing fecundity on HTL (Trial 1, $F_{1,102}=12.79$, $p=0.001$; Trial 2, ($F_{1,127}=31.68$,
107 $p<0.001$; Fig. 1). In this manner, performance of each individual was subsequently
108 estimated as its distance from the regression line. Thus, for any given body size, a negative

residual indicates a lower than average performance and a positive residual indicates higher than average performance. Performance of the individual lines/population was compared using General Linear Models and Tukey's multiple pairwise comparisons with line, trial and the interaction of line and trial as variables and residual fecundity as the response.

RESULTS

In total, 325 *T. pretiosum* were measured. HTL ranged from 0.103 to 0.172 mm, with a mean of 0.148 mm.

In the first study, comparing six inbred lines, mean residual fecundity was consistently highest in "high" performing isofemale lines, and lowest in "low" performing lines (line, $F_{5,201}=23.12$, $P< 0.001$; trial, $F_{1,201}=0.88$, $P< 0.350$; interaction of line and trial $F_{5,201}=7.99$; $P< 0.001$) , i.e. for any given body size, "high performing" lines (43 and 29) were more fecund than "low performing" lines (2 and 53) (Fig. 2). Surprisingly, in trial one, the least fecund line (for any given body size) was the "intermediate performing" line 9. Indeed, except for line 9, the hierarchical performance of the different lines did not change between trial one and trial 2 (performed 2 years and 78 generations later). However, while residual fecundity of the "low performing" lines (2 and 53) did not change significantly over this period, that of both intermediate lines (9 and 1) improved, and surprisingly, the residual fecundity of the "high performing" lines (29 and 43) deteriorated (Fig. 2).

130 In the second study, mean residual fecundity of the mixed population was close to
131 zero, as might be expected since the population was created as a mix of the previous six
132 inbred lines, and therefore may be considered a truly "average" population (Fig 2).
133 However, within this single population the correlation between wasp size and fecundity
134 was lost (Pearson correlation coefficient = -0.248, p=0.292).

135 In the third study, contrary to our expectations, prolonged rearing (2 v 40
136 generations) of a variable population did not result in an increase in the performance of that
137 population when controlling for differences in body size (i.e. residual fecundity)
138 ($F_{1,90}=0.00$, $P=0.962$), but overall fecundity did decrease ($F_{1,90}=13.76$, $P= < 0.001$)
139 (Figure 3).

140 DISCUSSION

141 A series of inbred isofemale lines exposes the genetic variation present in the field
142 population from which they were derived, and have been proposed as a means of
143 maintaining this variation over time (Hopper et al. 1993; Roush and Hopper 1995; Margan
144 et al. 1998; Nunney 2006). Inbred lines had been maintained for 150 generations since their
145 initiation and there were small fecundity differences among two trials conducted over two
146 years apart, in high and low performance. This information is useful to maintain quality
147 performance through time in mass-rearing facilities.

148 In the parasitoid wasp, *T. pretiosum*, female size influences female fecundity, with
149 larger females typically producing greater numbers of offspring (Fig. 2). However, there is
150 also a genetic component to fecundity, which was revealed in the present study by
151 examining the relationship between size and fecundity across several highly inbred

152 (isofemale) lines (Fig. 3). By effectively controlling for body size as a variable, we found
153 that for any given body size, females from some inbred lines were much more fecund than
154 others.

155 In field populations, genetic variation for morphological and life history traits, such
156 as tibia length and fecundity respectively, may mask any relationship between these traits
157 (Pavlik 1993; Kazmer and Luck 1995; Sorati et al. 1996; Ellers and Jervis 2003). However,
158 by maintaining isofemale lines, we were able to effectively remove genetic variation within
159 any particular line, while at the same time exposing the genetic variation (across multiple
160 different isofemale lines) that was originally present in a field population.

161 When the six inbred lines each with a clear correlation between size and fecundity
162 are mixed and reared for 2 generations, the correlation was lost. However, this is not
163 entirely surprising since "mixed-mating" introduces heterozygous offspring to the
164 population, some of which will have been sired by big wasps with low performance and/or
165 small wasps with high performance. This relationship also explains the differences in wasp
166 size and 48 h fecundity between genetically variable populations that have been reared for
167 two and forty generations. The differences can be explained through the heterozygosity in
168 the two generation or the loss of this, in the 40 generation population.

169 The isofemale lines methodology reduces the opportunity for adaptation to the
170 laboratory and may explain why in most cases the biological performance of inbred lines
171 did not change over time (with the exception of lines 43 and 9). Possible explanations for
172 this variation could be found in the sampling methods or the genetic variation of hosts.

173 More research can be performed along this issue to better understand isofemale lines
174 performance through time.

175 A remarkable finding is that it is possible to improve the parasitoid's size and
176 fecundity by means of directed hybridization of suitable inbred lines. The relationship
177 between size and fecundity offers a tool with which to monitor genetic variation in mass
178 rearing facilities. A strong positive correlation between size and fecundity may be
179 indicative of a population that has lost variation. Thus, we may be able to predict when is a
180 good time to introduce new variation and/or renew the entire mass-reared population.
181 Overall we conclude that isofemale lines methodology is useful to avoid domestication and
182 inbreeding depression, and the parasitoid's effectiveness as biological agent can be
183 enhanced.

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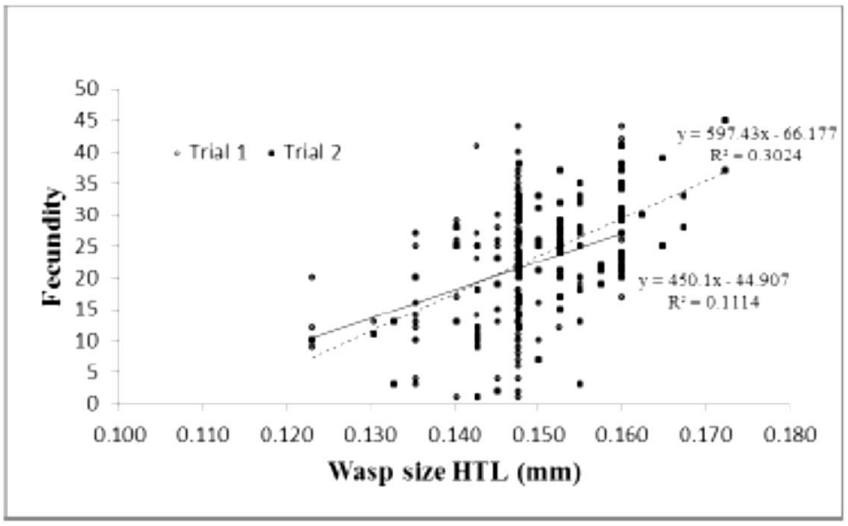
Figure 1 Relation between female size and fecundity, across six inbred *Trichogramma pretiosum* lines, in two trials separated by 76 (or 78) generations.

Figure 2 Relative fecundity in two trials, of six inbred *T. pretiosum* lines and a population created by mixing the six lines, controlling for the effect of female body size (see Fig. 1). Residuals were calculated by regressing fecundity on body size. Means followed by the same letter were not significantly different.

Figure 3 Performance of a *T. pretiosum* population, with initial high genetic variation, after 2 and 40 generations of laboratory-rearing, A) without, and B) with, controlling for differences in female size. The starting population was created by mixing 26 genetically different inbred lines.



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292 Fig 1

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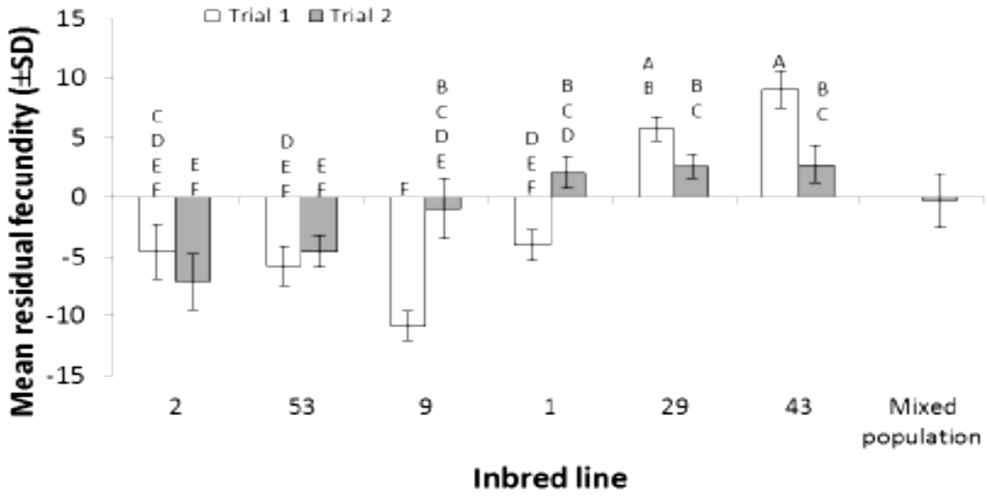
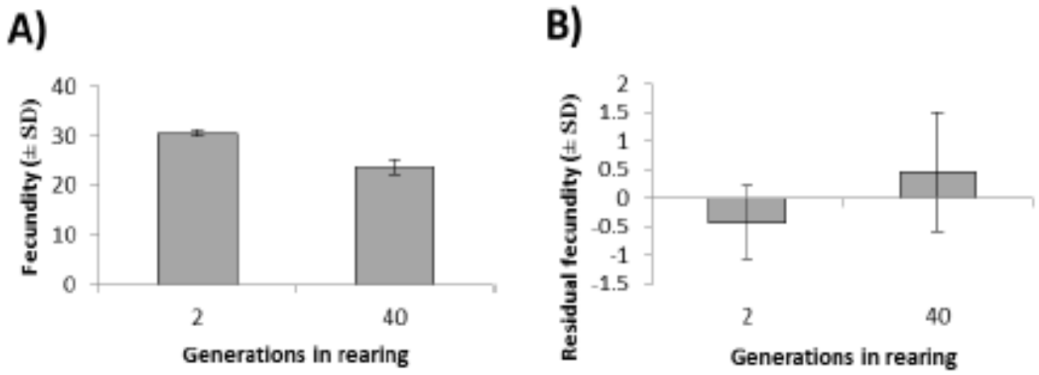

Trial 1
Trial 2
Mean residual fecundity (±SD)
15
10
5
0
-5
-10
-15
C D E F
E F
D E F
E F
F
B C D E
D E F
B C D
A B
B C
A
B C
2
53
9
1
29
43
Mixed population
Inbred line

Fig 2

299



300

301

302 Fig 3

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304

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Artículo científico III

DESEMPEÑO DE LÍNEAS PURAS Y LÍNEAS GENÉTICAMENTE VARIABLES DE *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) PERFORMANCE OF *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) PURE LINES AND GENETICALLY VARIABLE POPULATIONS

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RESUMEN

Mediante la comparación de sus parámetros poblacionales, fecundidad y tasa sexual, se evaluó el desempeño de 4 poblaciones de *Trichogramma pretiosum*: dos líneas puras altamente endogámicas (98%) con bajo y alto desempeño (2 y 43 respectivamente) y dos líneas genéticamente variables (producto de la combinación de 26 líneas puras) con 2 y 40 generaciones. Los resultados nos muestran que tanto en los parámetros poblacionales Ro, T, λ y rm, al igual que la fecundidad, la línea genéticamente variable con dos generaciones presenta los mejores resultados seguido de la genéticamente variable con 40 generaciones, después se ubica a la línea 43 y por último a la línea pura 2. A las 48 horas no existen diferencias significativas en la fecundidad entre las líneas genéticamente variables y la línea pura 43. A las 48 h, la proporción sexual no presenta diferencias significativas entre las dos líneas genéticamente variables y la línea pura 43, relación que cambia en la proporción sexual total, donde se observó un mayor número de machos en las líneas genéticamente variables.

Palabras clave: Parasitismo, parámetros poblacionales, endogamia, fecundidad, proporción sexual.

ABSTRACT

By comparing their population parameters, fecundity and sex ratio, we examined the performance of four genetically variable populations of *Trichogramma pretiosum*, two highly inbred pure lines (98%) with low and high performance (2 and 43 respectively) and two genetically variable lines (product of 26 inbred lines) with 2 or 40 generations. The population parameters determined were Ro, T, and λ as rm and fertility, and the overall results indicate significant differences between all the lines. The genetically variable line with two generations had the best performance, then the genetically variable with 40 generations, then line 43 and finally the pure line 2. After 48 hours, no significant differences were found in fertility between the genetically variable lines to pure line 43. A 48 h the sex ratio does not differ significantly between the two genetically variable lines and the pure line 43. But total sexual ratio showed higher number of males in the genetically variable lines.

Key words: Parasitism, population parameters, inbreeding, fecundity, sex ratio.

INTRODUCCIÓN

En programas de control biológico clásico o por aumento, mantener la variabilidad genética del enemigo natural es fundamental para que pueda adaptarse al medio ambiente (Chassain and Boulétreau 1991; Hopper et al. 1993; Wajnberg 2004). En ambos casos se necesita liberar gran cantidad de individuos, por lo que al incrementar el número de individuos en el proceso de reproducción en cautiverio afectamos su variabilidad, por lo tanto su capacidad, desempeño y calidad de la misma (Nunney 2003). Iniciar una población con pocos individuos puede generar un cuello de botella, al tener poca variabilidad genética que no refleja el total de alelos presentes en la población original (Hufbauer and Roderick 2005). Otro factor en la pérdida de variabilidad puede darse debido a que algunos individuos están mejor adaptados a las condiciones artificiales que otros, promoviendo la selección y/o eliminación de algunos alelos, dando lugar a un proceso

conocido como "domesticación" (Bartlett 1984; Allendorf 1986; Nunney et al. 2002), y esta eliminación de variabilidad puede conducir a un bajo desempeño en campo (Woodworth et al. 2002; Frankham 2005).

Para evitar la pérdida de variabilidad, algunos investigadores han sugerido subdividir la población de campo en líneas puras y posteriormente mezclar el mayor número de líneas puras (con un mínimo de generaciones) antes de liberarlas, para recuperar la variabilidad original, y de esta manera, evitar la domesticación (Roush and Hopper 1995; Kalyebi et al. 2005; Nunney 2006). Las líneas puras tienen de muy poca a ninguna variabilidad entre sus alelos, ya que son homocigotos para cada característica. Al no tener variabilidad que perder, no tienen cambios en el tiempo (excepto a través de mutaciones). Un ejemplo de su efectividad se puede observar en el uso de *Encarsia formosa*, 100% homocigota por su partenogénesis telitoquia, usada con éxito en Europa para el control de la mosquita blanca en invernaderos desde 1930 (van Lenteren et al. 1997). A pesar de las recomendaciones sobre el uso de líneas puras hay un número muy reducido de trabajos en parasitoides que prueben esta hipótesis (Sorati et al. 1996; Woodworth et al. 2002; Guzmán-Larralde et al. 2013) en donde se elijan y comparen líneas puras sin influencia del hospedero y poblaciones genéticamente variables con un número distinto de generaciones.

Para determinar y comparar el desempeño de líneas puras y poblaciones, y elegir aquella con mejor desempeño, se han utilizado distintos métodos. Uno de ellos son las tablas de vida de edad especifica, que son consideradas como una herramienta para estimar parámetros de crecimiento, desarrollo y reproducción, al incluir en su análisis, sobrevivencia, tiempo y proporción sexual (Lotka 1907; Lewis 1942; Leslie 1945; Southwood 1978; Bellows Jr et al. 1992; Maia et al. 2000; Badii and Castillo. 2009). Siendo utilizadas en investigaciones con *Trichogramma* (Pak and Oatman 1982; Pratissoli and Parra 2000; Haile et al. 2002; Pratissoli et al. 2004; Samara et al. 2008; Iranipour et al. 2009). Otros métodos relacionados con la evaluación de la calidad y desempeño en

parasitoides son aquellos que usan cálculos de fecundidad y proporción sexual (Waage and Ming 1984; Cerutti and Bigler 1995; Kazmer and Luck 1995; Sorati et al. 1996; Antolin 1999).

Por lo anterior el presente estudio tiene como objetivo comparar el desempeño y el efecto de la reproducción artificial de líneas puras y líneas genéticamente variables, para proporcionar evidencia de los beneficios de subdividir una población de campo, evitando la domesticación y manteniendo la variabilidad genética.

MATERIAL y MÉTODOS

Obtención del material biológico

Las líneas puras de Trichogramma pretiosum fueron colectadas en 2008, en campos de tomate de la Universidad de California (estación Irvine) en Estados Unidos y desarrolladas en huevos de *Manduca sexta*. Cada hembra colectada de manera individual fue reproducida con su propia progenie (hermanados) por nueve generaciones La líneas puras resultantes tienen un coeficiente de consanguinidad del 87% (Li 1955). Posteriormente se mantuvieron las colonias indefinidamente en el orden de cientos de individuos en *Ephestia kuehniella*. El experimento se realizó usando una incubadora a 25 °C, 16/18 horas (luz/obscuridad) a 30% de humedad relativa.

Comparación de las poblaciones

Se compararon cuatro poblaciones de manera simultánea. Para lo cual en un trabajo previo, se analizaron los parámetros reproductivos de 26 líneas puras (Guzmán-Larralde et al. 2013), donde para este trabajo se seleccionaron dos poblaciones, de las 26 líneas puras, una con alto y otra con bajo desempeño (nombradas como línea 43 y 2 respectivamente). Las otras dos poblaciones son una mezcla "genéticamente variable" (como resultado de la combinación de las 26 líneas puras, incluyendo a la 43 y 2) con dos generaciones (GV2) y con 40 generaciones (GV40).

De cada una de las poblaciones se usaron 25 hembras a 24 horas de emergencia de la población, estas se colocan individualmente en un tubo de cristal con un aproximado de 100 huevecillos de *E. Kuehniella* y una pequeña gota de miel. Cada 2 días (48 h) los huevecillos se renuevan y se colocan en viales separados, hasta su muerte, y la progenie se contabiliza y sexa.

Análisis matemático y estadístico

La comparación del desempeño se hizo mediante el cálculo de los parámetros poblacionales: Tasa neta de reproducción, ($Ro = \sum lxmx$), el número de hembras nacidas por cada madre, en cada generación; Tiempo generacional, ($T = \sum mxlx.x / \sum mxlx$) intervalo de tiempo medio, entre el nacimiento de los individuos de una generación y la de la próxima generación; Tasa finita de incremento ($\lambda = e^{rm}$) número de individuos que se agrega a la población por individuo y por tiempo; Tasa intrínseca de crecimiento, rm, ($\sum e^{-rm^x} lxmx = 1$) potencial de crecimiento de una población. El procedimiento para determinar la fecundidad a 48 horas, y total se llevó a cabo siguiendo la metodología descrita en el trabajo de Guzmán-Larralde et al. (2013), y el cálculo de la proporción sexual por medio del método descrito en Wilson and Hardy (2002). Los datos de fecundidad se compararon mediante un ANOVA usando Minitab para Windows (versión 16; Minitab Inc., State College, PA). Las medias con diferencias significativas fueron comparadas usando el método de Tukey. La tasa sexual fue transformada mediante arco seno raíz cuadrada, antes del análisis estadístico.

RESULTADOS

En la comparación del desempeño mediante parámetros poblacionales (Cuadro 1) se encontró para Ro, resultados que muestran valores que varían desde 12.52 para la línea pura 2, hasta la mayor tasa, 86.24 para la población GV2. El tiempo generacional (T) varía desde 13.8 para la línea pura 43 hasta 15.2 para la línea GV40. La tasa finita de crecimiento fue mayor a uno ($\lambda > 1$) para todas

las líneas, prediciendo un incremento sostenido en todas las poblaciones. Por ultimo rm se manifiesta entre 0.178 para la línea pura 2 y 0.327 para la GV2 (Cuadro 1).

Además se encontraron diferencias significativas en el análisis de fecundidad a las 48 h ($F_{3,82}$=13.50; $P < 0.001$) y en la fecundidad total ($F_{3,82}$=48.60; $P < 0.001$). El análisis de medias muestra dos grupos bien definidos para la fecundidad a las 48 h. Uno compuesto por la línea pura 2, que fue la que presentó los valores de fecundidad más bajos (19 ± 2.21) y otro compuesto por las tres poblaciones restantes; siendo la GV2 la que presentó los valores más altos de fecundidad (35 ± 1.64). Para la fecundidad total las medias muestran diferencias significativas entre cada una de las cuatro poblaciones comparadas, siendo la GV2 la que presento los valores más altos (215 ± 8.8). (Cuadro 1).

En las comparaciones de la tasa sexual se encontraron diferencias significativas en el análisis de varianza de las 48 h ($F_{3,82}$=15.04; $P < 0.001$) y en los datos totales ($F_{3,82}$=5.79; P=0.001). La comparación de medias a las 48 h muestra dos grupos bien definidos, uno con la proporción de macho más alta compuesto por la línea 2 y otro grupo por las tres poblaciones restantes; siendo la GV2 la que presentó una menor proporción de machos (0.14 ± 0.050). Así mismo los datos totales de la tasa sexual se forman dos grupos, siendo las que presentan una menor proporción de machos las líneas 2 y 43 (0.52 ± 0.001 y 0.48 ± 0.001 respectivamente), y la que tiene una mayor proporción de machos GV2 (0.78 ± 0.001) (Cuadro 2)

DISCUSIÓN

En la comparación del desempeño mediante los parámetros poblacionales, podemos observar diferencias entre las poblaciones genéticamente variables y las líneas puras (Cuadro 1). La combinación de un mayor número de líneas con menos generaciones, constituye la población con

mejor desempeño. Esto concuerda con estudios hechos dos años antes por Guzmán-Larralde et al. (2013), en un hospedero distinto, donde comparó poblaciones genéticamente variables con distinto número de generaciones, líneas puras y de laboratorios de reproducción masiva. Sin embargo es importante destacar que la fecundidad a las 48 horas no presenta diferencias entre las dos poblaciones genéticamente variables GV2 y GV40 y la línea 43, información que toma relevancia al valorar que en la naturaleza los individuos tienen una mayor presión de selección del ambiente, y por lo tanto, un menor tiempo de sobrevivencia comparada con la que se presenta en laboratorio de manera artificial. Una alta fecundidad durante los primeros días concuerda con estudios en T. *minutum y T. chelonis* cuando se provee un ilimitado número de huevecillos diariamente (Bai and Smith 1993; Miura and Kobayashi 1995). Además se pudo observar una reducción de las tasas poblacionales y de la fecundidad total de la línea genéticamente variable GV40, al incrementar el número de generaciones, lo que sugiere una reducción del desempeño de la población a través del tiempo. Esta información coincide con los estudios hechos por Woodworth et al. (2002) en donde encontró un deterioro genético y una reducción en el desempeño en poblaciones de Drosophila después de 50 generaciones en condiciones de laboratorio. Por lo tanto, si queremos reducir el costo de la renovación de la variabilidad genética de la población en cautiverio, una alternativa sería utilizar una línea pura con alto desempeño en laboratorio y campo.

Lo más deseable en una producción de parasitoides es un mayor número de hembras, ya que estas influyen directamente en la reducción del hospedero, por lo tanto una menor proporción de machos, le confiere ventajas a la población. La proporción de sexos a las 48 h, señala a las poblaciones genéticamente variables GV2 y GV40 y la línea pura 43, como aquellas que producen un mayor número de hembras. Sin embargo esta relación cambia radicalmente en el balance total en el caso de las dos líneas genéticamente variables, pero no así en la línea 43, quien mantiene su condición basada en hembras (0.48 ± 0.001). La proporción sexual encontrada en hembras de esta misma línea 43 en un experimento anterior, Febrero 2011 (Guzmán-Larralde et al. 2013), y la

encontrada en este estudio (Mayo 2013), con más de 2 años entre ellos y aproximadamente 95 generaciones, se mantuvo constante, lo que le confiere ventajas al uso de líneas puras, esto concuerda con la investigación de Antolin (1999) quien recapitula distintos trabajos con especies puras haplodiploides, incluyendo a *T. pretiosum*, y en donde no encuentra cambios en la proporción sexual.

Es indudable que aún falta investigación acerca del desempeño en campo, usando líneas puras exitosas en estudios de laboratorio. En resumen, subdividir una población de campo en líneas puras asegura la permanencia de ciertas características (alelos) en el tiempo y combinar las mejores líneas dependiendo del desempeño específico a condiciones climáticas y biológicas, previo a su liberación, promueve ventajas en los resultados que los centros de reproducción masiva buscan, incrementando y manteniendo las características deseadas a través de innumerables generaciones.

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CONCLUSIONES

Como resultado de la creación de líneas puras a partir de una misma población, la variabilidad genética se vio expuesta en 26 líneas distintas, a través del análisis de sus parámetros biológicos, y éstos se mantuvieron relativamente constantes a través de 95 generaciones. La metodología de líneas puras reduce la oportunidad de adaptación al laboratorio de reproducción masiva.

En la combinación de líneas puras con distintos parámetros biológicos, basados en el rm, siempre se observó un incremento, pero éste se suscribía al rango máximo de las líneas involucradas.

Después de la emergencia de la avispa, en los primeros días, la tasa sexual muestra un mayor número de hembras que de machos, y esta cantidad decrece con el tiempo para producir más machos en los últimos días. Observamos que en promedio del 50 al 77 % de todas las líneas produce la mayoría de las hembras hasta los días 6 y 8. Por lo que la forma más eficiente de producir un parasitismo con porcentajes altos de hembras sería permitiendo a las hembras el parasitismo solo en los primeros días después de su emergencia con un maximo de seis días.

Se encontró una relación positiva entre la variabilidad genética, tamaño y fecundidad en líneas puras. Que se perdió al combinar estas mismas líneas puras en las primeras 2 generaciones. Poblaciones variables genéticamente (combinando 26 líneas puras) con 2 generaciones, reducen la relación entre tamaño y fecundidad. Sin embargo al incrementar el número de generaciones, la relación se incrementa, quizás por una selección de ciertos alelos. Por lo que es muy probable que una relación positiva en tamaño y fecundidad en poblaciones de laboratorio indiquen una pérdida de variabilidad.

Los resultados mostraron que una línea pura (con al menos 96 generaciones) con un rm alto, es comparable a una población formada por la mezcla de 26 (con 2 generaciones). Por lo que una solución simple sería seleccionar líneas puras con buen desempeño, evitando la domesticación, producto de la reproducción repetida en cautiverio.

En la comparación de poblaciones genéticamente variables, iguales pero con distinto número de generaciones (dos y 40) se observaron diferencias significativas en su fecundidad. Por lo que las poblaciones genéticamente variables, colectadas directamente del campo o producto de la combinación de varias líneas puras, tendrán ventajas en su desempeño en sus primeras generaciones, comparadas con aquellas mantenidas por largos periodos en cautiverio.

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