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(54) **Title**: SYSTEM OF BREEDING BUMBLEBEES FOR IMPROVED MONITORING AND INCREASED POLLINATION EFFICIENCY

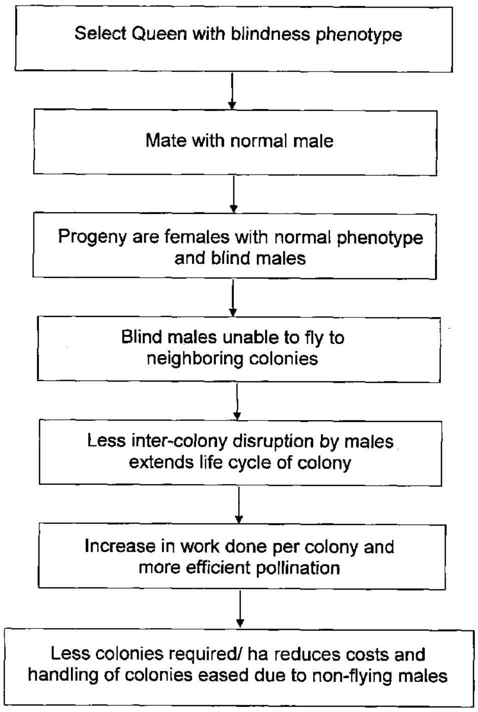

FIG. 3

(57) **Abstract**: A system useful for pollinating a field comprising an artificial domicile essentially devoid of normal males, said domicile inhabited by a bee population comprising at least one phenotypically blind and white eyed queen bee characterized by homozygosity for blindness (b) and white eye, a plurality of female phenotypically wild type offspring characterized by heterozygosity for blindness and white eye and a plurality of phenotypically blind and white eyed males characterized by blind and white eyed co inherited alleles.

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SYSTEM OF BREEDING BUMBLEBEES FOR IMPROVED MONITORING AND INCREASED POLLINATION EFFICIENCY

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to breeding of bumblebees and particularly to methods of generating bumblebee colonies with improved pollination efficiency and ease of monitoring the pollination activity in the field.

Background Art

The cultivation of bees is an integral part of modern agriculture as they are the main pollinators of flowering plants. It is estimated that a third of the human food supply is dependent on insect pollination, mostly by bees. The honey bee (*Apis mellifera*) is the most common pollinator used in agriculture, mostly due to the large size of perennial colonies (up to 40,000 worker bees). Bumblebees (the genus *Bombus)*, leafcutter bees (Megachile) and a few other species are also used as pollinators, but because of their small and short-lived colonies (bumblebees), or their complex management system (leafcutter bees), their cost is high and they are used only on those crops where they have an advantage over the use of honeybees. A few examples of such crops are: tomatoes, blueberry and alfalfa for seeds.

Tomatoes and a few other important crops such as blueberries, cranberries and eggplants (about 8% of crops worldwide) have anthers with small apertures. The pollen in these plants is deep inside and firmly attached and while shaking by wind may be sufficient to dislodge the pollen and to release it through pores in the anther, buzz pollination by bumblebees and a few other species of solitary bees is far more efficient. During buzz pollination, the bumblebee grabs hold of the flower with its legs or mouth and moves its flight muscles rapidly, but without moving its wings, creating a resonant vibration, which dislodges the pollen. The electrostatically charged pollen is then attracted to the oppositely charged body hairs on the bumblebee. The bumblebee then grooms the pollen into structures on its back legs and abdomen and transports it back to its nest. While bumblebees and many other species of solitary bees can collect pollen from these type of flowers using the method of buzz pollination, honeybees are unable to do so, and they will leave the flowers after short time of unsuccessful attempt to extract the pollen.

Although greenhouse tomatoes are autogamous, pollination is necessary in order to achieve good enough fruit set. Studies have shown that an average of 2,000 bee trips per hectare per day

(Morandin et al., 2001) was adequate to ensure sufficient pollination and could be achieved by placing between 7 and 15 colonies per hectare, evenly distributed throughout the greenhouse.

In the past greenhouse tomatoes were traditionally pollinated using handheld electric vibrators such as the "Electric Bee", but it has been found that using bumblebees is both more cost effective and less time-consuming than the use of electric vibrators and also crucially, causes less damage to the plants themselves. Consequently the use of bumblebee pollinators rather than mechanical pollinators decreased pollination costs by about 10% per hectare.

Current practice uses bumblebees as the most efficient pollinator of tomato plants. However, the cost of pollination by bumblebees is relatively high (although still makes economical sense) when compared to the cost of pollination by honeybees in other crops (such as melons, cotton, sunflowers, pepper). The main reason for the high cost of using bumblebees is their small annual colonies.

A bumblebee colony (a family) rapidly develops from a single queen to a fully developed family containing about 200 workers and then collapses when all the workers die, this whole cycle being completed within 5 months. Thus, to ensure adequate pollination, for a given area, more bumblebee colonies are needed (then honeybees). Moreover, if the pollination period has to last more than 6 weeks, a new set of colonies will be needed, as the first batch of colonies will die after 6 to 8 weeks in the field. Any disturbance or stress that a colony experiences, such as invasion by bees from other colonies, may have a dramatic effect on its development and shorten its life in the field, resulting in overall more colonies used in a given area during the pollination period.

RU 2149540 C1 introduces a method of raising high-productivity bumblebees taking male bumblebees as population improvers and mating them with Queens with improved features to obtain bumblebees with increased reproductive and flying capacity, optimum development and improved adaptation to a wide range of temperatures and humidity conditions. While this invention also uses bumblebee reproduction to improve productivity of bumblebees, it does not address the problem of inter-colony interference. US 6010390 introduces a process that utilizes a clean manufacturing environment to rear insect ovum, including bumblebees to imagoes that are isolated from agents of disease, predation, and parasitism. This pollination method employs a point-to-point distribution system that also substantially reduces material, handling, and shipping costs. Again this system does not address the problem of invasion of young colonies by mature male bumblebees that disrupt the colonies and have no function for agricultural pollination purposes.

Thus it is a long felt need in the field of agriculture to have a stable system of pollination in a field or greenhouse that is both efficient and cost effective. The present invention solves many of the problems associated with pollination in the agricultural industry by introducing an artificial domicile devoid of normal males.

SUMMARY OF THE INVENTION

It is one object of the present invention to disclose a system useful for pollinating a field or greenhouse that comprises an artificial colony essentially devoid of normal males, with the colony being inhabited by a bee population comprising of at least one phenotypically blind and white eyed functioning queen bee characterized by homozygosity for blindness and white eye, a plurality of female workers that are phenotypically wild type offspring characterized by heterozygosity for blindness and white eye, a plurality of phenotypically blind and white eyed males characterized by blind and white co inherited alleles and a minority of phenotypically normal males, constituting not more than 10% of the male population, wherein the artificial colony system has an improved pollination effectiveness. It is also in the scope of the present invention to disclose an artificial colony system wherein the population is derived from *Bombus terrestris* carrying mutated alleles.

It is also in the scope of the present invention to disclose an artificial colony system comprising a plurality of phenotypically blind and white eyed functioning queen bees characterized by homozygosity for blindness and white eye, and a plurality of phenotypically normal males, so as to provide bees as a breeding population useful for the propagation and population of said artificial colony system.

It is also in the scope of the present invention to disclose a queen bee useful for populating an artificial colony system, the queen being characterized by homozygosity for a heritable recessive navigational impairment trait (*nit*) and an eye pigment trait (*ept*).

It is also in the scope of the present invention to disclose that *nit* and *ept* are co-inherited on mutant allele **b**. it is also in the scope of the present invention wherein non-impaired navigational skills and *ept* are co-inherited on allele **P**.

It is also in the scope of the present invention to disclose that the phenotypically normal female workers produce a subpopulation of normal males without mating.

It is also in the scope of the present invention to disclose a queen bee useful for populating an artificial colony system, the queen being characterized by homozygosity for *nit* and *ept*, wherein the haploid male offspring of said queen exhibit non disruptive colony behaviour (NDCB).

It is also in the scope of the present invention to disclose a queen bee useful for populating a pollination colony, homozygotic for heritable recessive navigational impairment traits (*nit*), the queen being characterized by male offspring having an eye pigment trait (EPT) defined by eye pigment intensity less than the measurement obtained from normally pigmented bees, measured at a standardized wavelength and under standard conditions, wherein said EPT is co inherited with *nit*.

It is also in the scope of the present invention to disclose an artificial colony system, useful for pollinating a field or greenhouse crop, characterized by male offspring exhibiting NDCB.

It is also in the scope of the present invention to disclose a field or greenhouse crop, populated by the artificial pollination colony system wherein the crop is preferably but not exclusively selected from tomatoes, strawberries, blueberries or any other artificially cultivated crop.

It is another object of the present invention to disclose the artificial colony system, wherein pollination efficiency is increased by 10%.

It is another object of the present invention to disclose a method for crop pollination comprising; placing in a field or greenhouse of plants to be pollinated a plurality of bumblebee colonies, wherein each colony is inhabited by a queen capable of producing males exhibiting NDCB and allowing the bumblebee colonies to forage among and pollinate the plants.

It is also in the scope of the present invention to disclose a method of crop pollination by a bee population in an artificial colony system comprising: providing a plurality of rearing units located within an appropriate environment isolated from substantially damaging or lethal external factors, providing said rearing units with the appropriate diet, populating each of said plurality of rearing units with one phenotypically blind and white eyed functioning queen bumblebee characterized by homozygosity for blindness and white eye and populating said rearing units with a plurality of phenotypically normal male bumblebees wherein the bumblebees present are provided as a breeding population useful for the propagation and population of the artificial colony system.

It is also in the scope of the present invention to disclose a method, wherein the NDCB males are unable to orient outside their colony. It is also in the scope of the present invention to disclose a method, characterized by non-invasion of NDCB males of the plurality of colonies in the field or greenhouse, wherein the working life-cycle of a plurality of colonies is extended.

It is also in the scope of the present invention to disclose a method, useful for pollinating a crop wherein the crop is preferably but not exclusively selected from tomatoes, strawberries, blueberries or any other artificially cultivated crop.

It is also in the scope of the present invention to disclose a method, wherein the overall pollination efficiency of the colonies in the field or greenhouse is increased by not less than 10%.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic showing the dynamics of colony development.

FIG. 2 is a schematic showing production of appropriate progeny from F1 to F4 generation.

FIG. 3 is a simplified flow chart of a method for pollination in accordance with a preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference is now made to fig. 1, illustrating the dynamics of colony development. In one embodiment of the present invention bumblebees are characterized by a specific dynamic of colony (family) development (described by Duchateau and Velthuis, 1988). The timeline (60) of the growth of a bumblebee colony (or family) goes through 3 phases. The first phase is the "solitary phase" (62) which starts when the queen lays her first egg (61). During this phase, the queen is alone and has already mated, storing the sperm in the spermatheca deciding to fertilize the eggs as she is laying them, but has no workers to help her in caring for the eggs and larvae. During this phase, the queen lays fertilized eggs that will normally develop into female workers.

The second phase of colony development starts when the first workers emerge (64). This event marks the beginning of social life (63). From this point onward, there will be more and more adult bees in the colony. The duration of the second phase lasts about 4 weeks in which during the first 2 or 3 weeks of the second phase, the mother queen continues to lay fertilized eggs that will develop into females (diploid, having chromosomes from the queen as well as from the male). However, at some point during the second phase of colony development, the queen switches to laying unfertilized eggs that will develop into males (haploid with chromosomes from the queen only). The switch from laying fertilized to unfertilized eggs is a very important social event called the "switch point". The second phase of colony development ends about 4 weeks after the beginning of social life (emergence of the first worker), when some of the old workers in the colony start to lay their own eggs and destroy the eggs laid by the queen.

The beginning of egg laying by workers is called the "competition point" (65) and it marks the beginning of the competition phase (66). The competition point also marks the collapse of the social order in the colony. From this day on, many workers will start to lay eggs but most of them will be destroyed by the queen and by other workers who try to achieve their own dominant status. Female larvae younger than 3 to 5 days when the competition phase start will develop into young queens instead of workers. Female larvae exposed to a pheromone in the first five days of larval life will become workers. The competition phase lasts for 6 to 10 weeks. It should be noted that in some cases the queen can continue to lay a mix of fertilized and unfertilized eggs after the switch point and even during the competition phase.

In the first 2 or 3 weeks of this phase, the number of adult workers in the colony will increase due to the emergence of workers from fertilized eggs laid by the queen before the competition phase started. The cumulative number of workers (70) begins to increase more rapidly during this phase. However, after peaking at about 2 to 3 weeks into the competition phase, the number of adult workers in the colony starts to decrease (due to mortality). The queen will die at some point between the beginning of the competition phase and 4 weeks later. About 8 weeks after the competition phase begins, bumblebees in the colony will cease to function as a family. The queen and most of the workers are already dead, young queens (90) and males (80) emerge and leave the colony to find a mate.

Bumblebee colonies are introduced into a greenhouse (100) when they have about 50 to 70 adult workers. This usually happen about 3 to 4 weeks after the emergence of the first worker, and at about the time of the competition point.

The advantage of introducing the colony at this time is that it already has enough workers who can take care of the brood and collect pollen from flowers. Colonies that are introduced into the greenhouse at younger age are not stable enough and many do not survive. Introduction of colonies at an older age has two disadvantages:

1. they have less time to live and function in the greenhouse.
2. they suffer stress due to the increased number of workers inside the closed hive prior to the introduction.

Besides the 50 to 70 adult workers, the colony contains the mother queen, a few dozens of larvae and pupae of workers, some young female larvae that will develop into queens and unfertilized eggs that will develop into males.

In the first 3-4 weeks in the greenhouse, the number of workers in the colony increases and they collect pollen to feed the young larvae in the colony (most of them will develop into young queens and males). When the adult males emerge from their cocoons, they spend a few days inside the colony and then fly outside to find a mate. Since there is no nectar in tomato flowers, males can feed only inside the hive.

For pollination purposes, it is best to use colonies in which worker production continues for as long as possible. In other words, the switch point and the competition point are very late (the queen lays a high number of fertilized eggs and most of these eggs develop into workers).

The timing of these social events is a crucial factor in selecting high quality colonies (for pollination purposes). It is believed that the timing of the switch point is innate to the queen (it is a character of the queen that is not affected by external factors).

However, the dynamic of colony development can be also affected by external factors (not directly related to bee genetics). Food quality and availability, adults population density in the hive, the size of the domicile, ambient temperature, parasites and diseases are a few examples. Excessive numbers of adult workers and males intruding on the brood (the brood includes the eggs, larvae and cocoons), can have a negative effect on larvae survival and impose stress on the queen. Thus, reducing the number of adult bees (workers and males) on the brood can contribute to prolonging colony development. More broods will survive for a longer time and will require more pollen (as food) that will be brought in by workers.

The workers forage for food for the rest of the colony and in so doing pollinate the crops in the greenhouse. The males and young female larvae do not contribute to the pollination of the crops outside the colony. The workers life span is usually about 8 to 12 weeks, during which time the worker population of the colony will increase to a peak of approximately 200 live individuals 3-5 weeks after introduction of the colony. Thus it is clear that the adult males in the colony serve no useful purpose for agricultural purposes of pollination.

In the present invention, an artificial colony system is presented, populated by normal bumblebee workers and blind males easily identifiable by their white eyes. Such blind males are unable to orient themselves outside the hive and thus unable to fly around in the greenhouse and disturbing other colonies.

Another aspect of the invention is a bumble bee with mutant allele, b, which is recessive and in its homozygous form results in bumblebees that are blind and easily identifiable by their white eyes, bumblebees carrying the normal allele, P, which is dominant, have normal pigmented brown/black eyes. In another aspect of the present invention the wavelength measurement of the eyes of the mutant bees can be measured to be significantly different to the measurement of pigmented normal bumblebee eyes.

The term "artificial colony system" refers to a man-made colony system for bees, comprising at least one but preferably a plurality of colonies, populated by specially selected bees for pollination purposes.

One of the major factors affecting the longevity and efficiency of a colony is the density of adult bees (female and males) on the brood. Invasion of young colonies by males from mature colonies can disturb the Queen and her workers, shortening the life of the colonies and thus increasing the number of colonies needed for maintaining adequate pollination level in a given greenhouse area.

Introduction of the mutation suggested in this invention would mean that all the males would be blind and unable to fly around and consequently unable to disturb other colonies.

In addition the inability to fly of these mutated bumblebees would make it easy to monitor bumblebee activity in the greenhouse. In greenhouses containing normal colonies, it is almost impossible to tell what proportion of the flying bees are workers and what proportion are males (who do not contribute to pollination). It is possible to see large number of bees flying in the greenhouse but if most of them are males, there will be no pollination activity..

Since the mutation is recessive fertilization of a blind queen with a normal male would result in progeny where all females are phenotypically normal and all the male progeny is blind since males are haploid and would only be able to inherit the recessive gene from the blind queen. Thus the colony would consist of workers that are able to carry out their normal work and males that would be unable to disrupt other colonies. This would greatly increase the efficiency and longevity of the all the colonies.

Reference is now made to fig. 2 which illustrates the mating methods used to generate the line of mutated bumblebees with the desired phenotype in accordance with one embodiment of the present invention. In this simplified schematic P=pigmentation, representing the normal allele and b=blindness, representing the mutant recessive allele. A first generation (10) of mating bees is introduced, preferably including at least one male (2) with the recessive mutant allele b, and at least one female queen (3) homozygous for the normal allele P. The progeny (20) of the first mating pair, also known as the F1 generation would then preferably include a plurality of each of the following, normal males (4) with the P allele, phenotypically normal heterozygous (herein denoting females carrying both the P and b alleles) workers (5) and phenotypically normal heterozygous queens (6).

In accordance with the present invention, heterozygous queens of the F1 generation are mated with normal males (7). Progeny of the F2 generation (30), should preferentially but not exclusively include a plurality of males with the mutant b allele (2), normal males (4), phenotypically normal homozygous (8) and heterozygous (9) workers, homozygous normal queens (3) and phenotypically normal heterozygous queens (6).

In another embodiment of the present invention, the heterozygous queens of the F2 generation are mated with mutant males (2) carrying the b allele only to produce the progeny of the F3 generation (40). The F3 generation should preferentially but not exclusively include both mutant (2) and normal (4) males, phenotypically normal heterozygous workers (9), blind homozygous workers (11) and phenotypically normal heterozygous queens (6). It is an embodiment of the present invention that the F3 generation should preferentially include homozygous blind queens

(12) that are necessary for the generation of the colony system introduced by the present invention. These blind queens are then mated with a normal male (7) to produce the F4 progeny (50) of the novel colony system.

It is an embodiment of the present invention that the F4 generation should preferentially include only males carrying the mutant b allele (2), phenotypically normal heterozygous workers (9) and queens (6). Thus in the F4 generation, progeny are suitable for inhabiting an artificial domicile or colony to carry out the work of pollination and in which the population is devoid of normal males.

In fig. 3 a simplified flow chart of a method for bumblebee pollination in which artificial colonies are essentially devoid of normal males, illustrates the main embodiment of the present invention.

It is an embodiment of the present invention that females with the blindness phenotype, homozygous for the b mutant allele are generated through selective mating and selected for mating purposes for generation of artificial bumblebee domiciles devoid of normal males. In another embodiment of the present invention the blind queen is mated with a normal male, carrying only the normal P allele. Thus haploid males of the next generation can only inherit the mutant b allele from the queen and are blind. Females of the next generation must inherit one normal P allele from the male and a mutant b allele from the queen and are phenotypically normal.

The term "essentially devoid" refers to a colony of bumblebees in which the subpopulation referred to is no more than 10% of the population described therein.

It is an embodiment of the present invention that progeny in the artificial domicile used for pollinating fields and greenhouses consists of phenotypically normal queens and workers and devoid of normal males. In another embodiment of the present invention blind males of the artificial colony are unable to fly and therefore do not enter other colonies or return to their home colony after leaving it. In this embodiment they can leave the colony but cannot enter other colonies. In this embodiment disruption of colonies by foreign males, especially of young colonies does not occur and substantially increases the working life of the colony from 6-8 weeks by up to 2 weeks to 8-10 weeks.

Pollination effectiveness is defined as a measure of an insect's potential as a pollen vector, as reflected by its ability to effect fruit production significantly and pollination efficiency is defined as the relative ability of an insect to pollinate flowers effectively, as measured by fruit production per unit measure eg. per visit or trip.

Thus it is an embodiment of the present invention that the pollination effectiveness of the bumblebee colony overall will be increased as a consequence of increased pollination efficiency and prolonged working life-cycle of the colony.

It is another embodiment of the present invention that less inter-colony disruption also results in more developing brood that require more pollen to be collected by workers as food for larvae. Thus, increasing the work done per colony by 10-20%. It is an embodiment of the present invention that the number of colonies essentially devoid of normal males required per hectare (ha) during the growing season for pollination of a field or greenhouse is less than the number required if using normal colonies.

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FOREIGN PATENT DOCUMENTS

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CLAIMS:

1. A system useful for pollinating a field or greenhouse comprising
an artificial colony essentially devoid of normal males, said colony inhabited by a bee population comprising
   a. One phenotypically blind and white eyed functioning queen bee characterized by homozygosity for blindness and white eye,
   b. a plurality of female workers that are phenotypically wild type offspring characterized by heterozygosity for blindness and white eye
   c. a plurality of phenotypically blind and white eyed males characterized by blind and white co inherited alleles.
   d. a minority of phenotypically normal males, constituting not more than 10% of the male population.

   wherein said system has an improved pollination effectiveness.
2. A system according to claim 1, wherein the population of said artificial colony is derived from *Bombus terrestris* carrying mutated alleles.
3. The system according to claims 1-2, comprising an artificial colony system populated by:
   a. a plurality of phenotypically blind and white eyed functioning queen bees characterized by homozygosity for blindness and white eye.
   b. a plurality of phenotypically normal males.

   wherein the bees present are provided as a breeding population useful for the propagation and population of said artificial colony system.
4. A queen bee according to claim 1, useful for populating said artificial colony system, said queen characterized by homozygosity for;
   a. a heritable recessive navigational impairment trait (*nit*).
   b. an eye pigment trait (*ept*).

   wherein said *ept* and said *nit* are co inherited.
5. The queen bee according to claim 4, wherein *nit* and *ept* are co-inherited
on mutant allele **b**.
6. The queen bee according to claim 4, wherein non-impaired navigational skills and normal eye pigment are co-inherited on allele **P**.
7. The phenotypically normal female workers, according to claim 1, wherein said female workers produce a subpopulation of normal males, without mating.

8. A queen bee useful for populating a pollination colony, said queen characterized by homozygosity for *nit* and *ept*, wherein the haploid male offspring of said queen exhibit non disruptive colony behaviour (NDCB).
9. A queen bee useful for populating said artificial colony system, homozygotic for heritable recessive navigational impairment traits (*nit*), said queen characterized by male offspring having an eye pigment trait (EPT) defined by eye pigment intensity of less than the measurement obtained from norms measured at a standardized wavelength under standard conditions, wherein said EPT is co inherited with *nit*.
10. The artificial colony system according to claim 1, useful for pollinating a field or greenhouse crop, characterized by male offspring exhibiting NDCB.
11. The field or greenhouse crop according to claim 10, populated by the artificial colony system wherein said crop is preferably but not exclusively selected from tomatoes, strawberries, blueberries or any other artificially cultivated crop.
12. The system according to claims 1-10, wherein pollination efficiency is increased by 10%.
13. A method of crop pollination by a bee population in an artificial colony system comprising:
    a. providing a plurality of rearing units located within an appropriate environment isolated from substantially damaging or lethal external factors.
    b. providing said rearing units with the appropriate diet.
    c. populating each of said plularity of rearing units with one phenotypically blind and white eyed functioning queen bumblebee characterized by homozygosity for blindness and white eye.
    d. populating said rearing unit with a plurality of phenotypically normal male bumblebees.

    wherein the bumblebees present are provided as a breeding population useful for the propagation and population of said artificial colony system.
14. A method for crop pollination comprising:
    placing in a field or greenhouse of plants to be pollinated a plurality of bumblebee colonies, wherein each colony is inhabited with a queen capable of producing males exhibiting NDCB and allowing said bumblebee colonies to forage among and pollinate said plants.
15. A method according to claim 14, wherein said NDCB males are unable to orient outside their colony.
16. A method according to claim 15, characterized by non-invasion of NDCB males of the plurality of colonies in the field or greenhouse, wherein the working life-cycle of a plurality of colonies is extended.

17. The method according to claim 14-16, useful for pollinating a crop wherein said crop is preferably but not exclusively selected from tomatoes, strawberries, blueberries or any other artificially cultivated crop.
18. The method according to claim 14-17, wherein the overall pollination efficiency of the colonies in said field or greenhouse is increased by not less than 10%.

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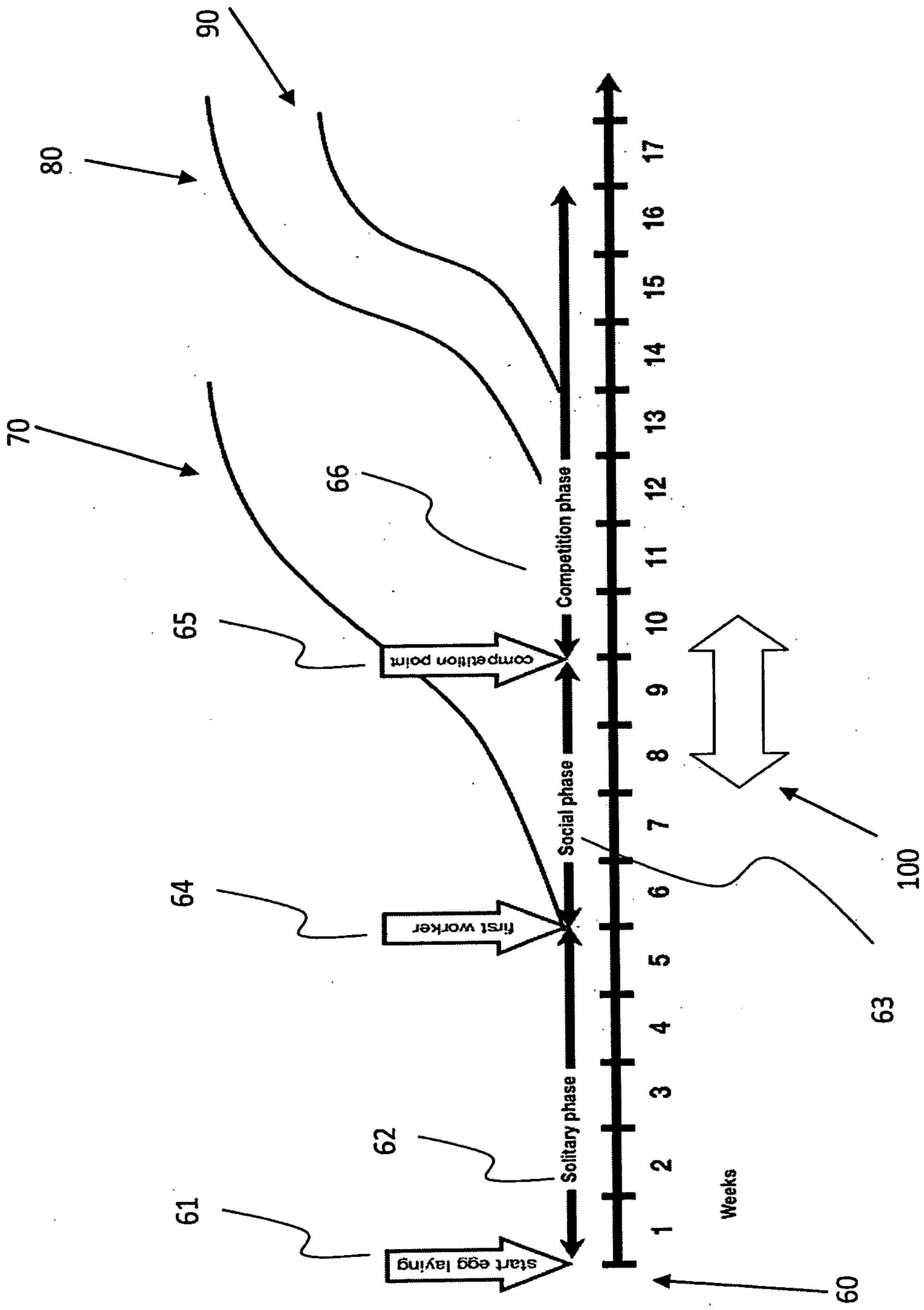
start egg laying
first worker
competition point
Solitary phase
Social phase
Competition phase
Weeks
1
2
3
4
5
6
7
8
9
10
11
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15
16
17
60
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66
70
80
90
100

FIG. 1

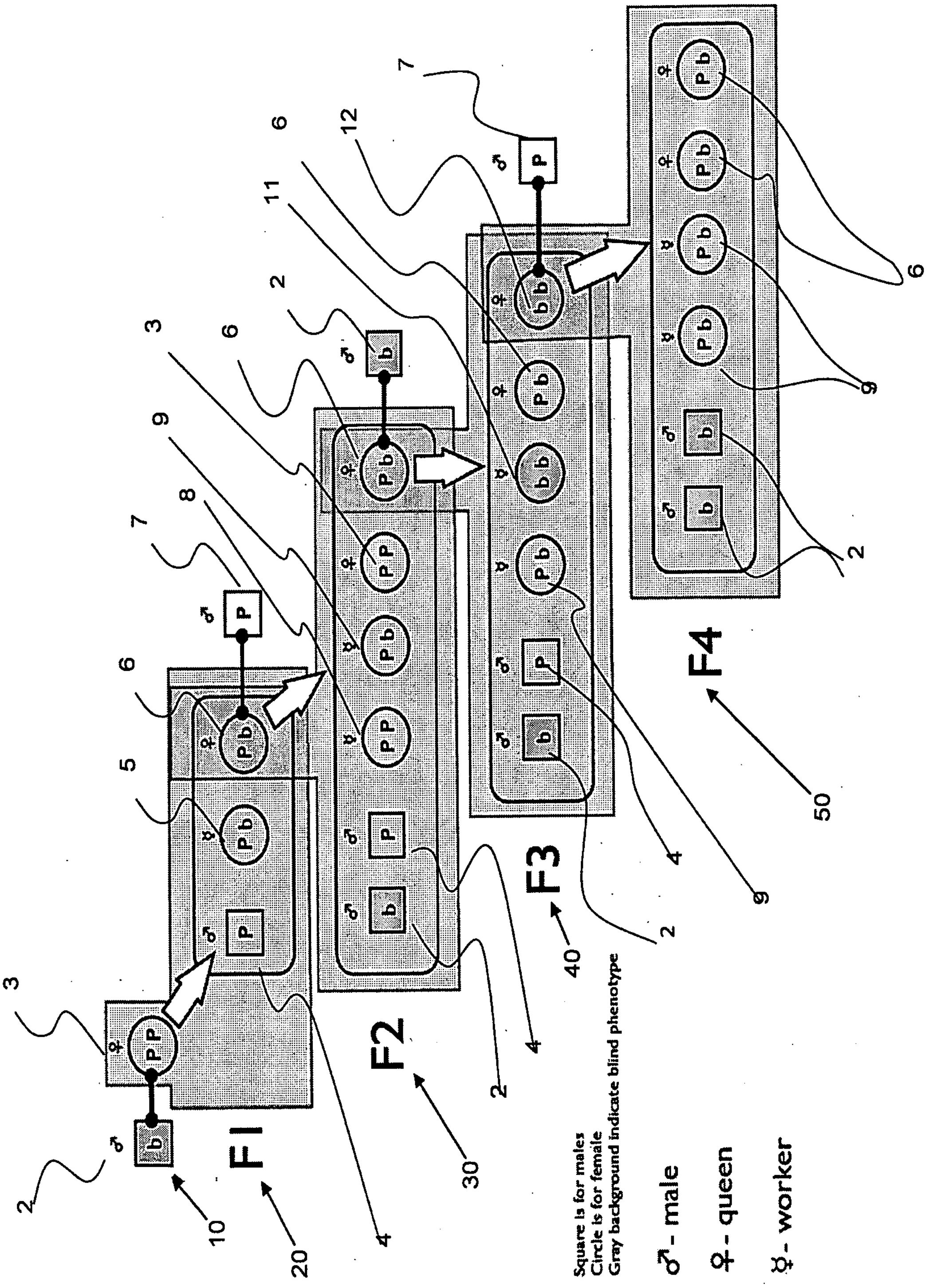
F1
F2
F3
F4
Square is for males
Circle is for female
Gray background indicate blind phenotype
♂- male
♀- queen
☿- worker

Figure 2

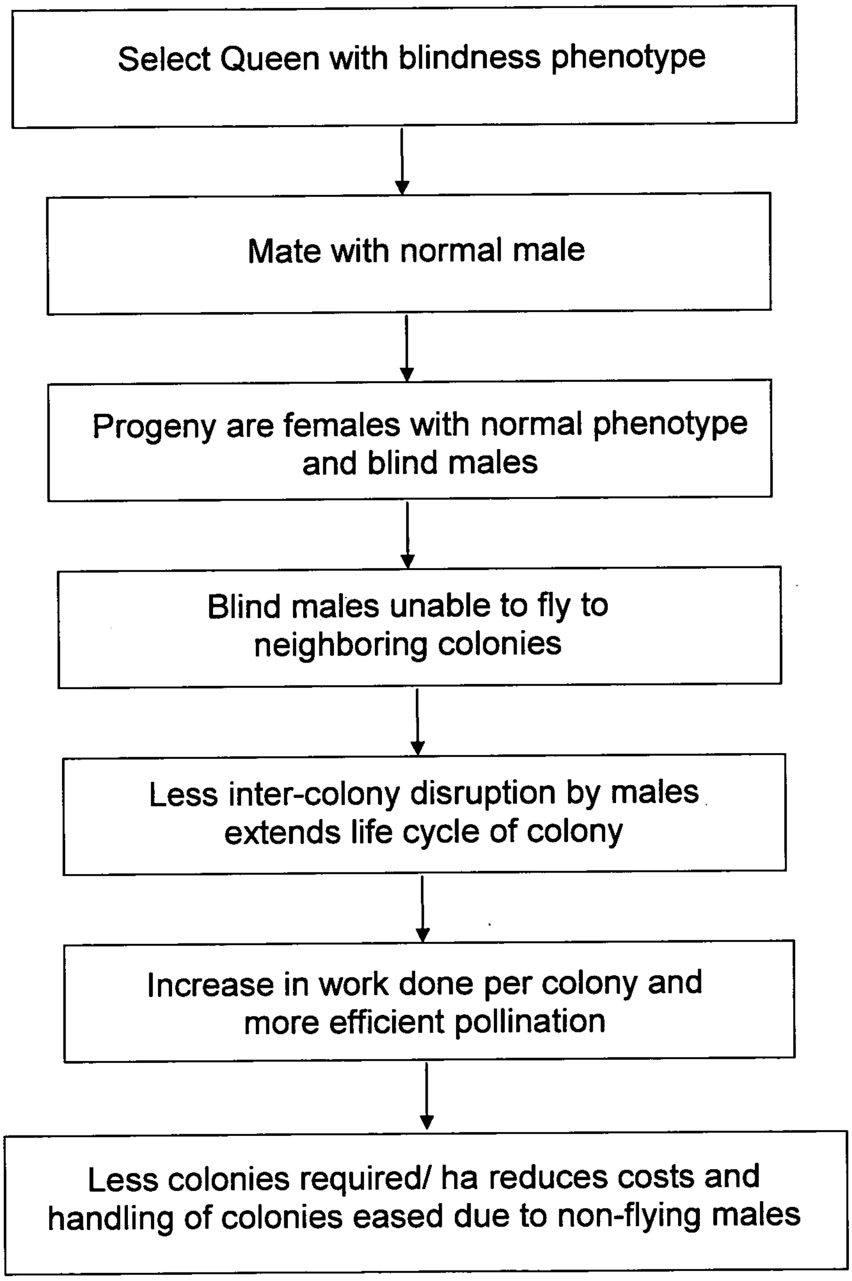
Select Queen with blindness phenotype
Mate with normal male
Progeny are females with normal phenotype and blind males
Blind males unable to fly to neighboring colonies
Less inter-colony disruption by males extends life cycle of colony
Increase in work done per colony and more efficient pollination
Less colonies required/ ha reduces costs and handling of colonies eased due to non-flying males

FIG. 3