

The Taxonomy of
Jaltomata tlaxcala and *Jaltomata procumbens* (Solanaceae):
Morphological Distinctions and Interfertility

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An Abstract of a Thesis
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ABSTRACT

The genus *Jaltomata* of the nightshade family (Solanaceae) currently contains various unresolved taxonomic and phylogenetic questions at the species level. A new taxon designated *Jaltomata tlaxcala* (nom. ined.) is closely related to *Jaltomata procumbens* (Cav.) J. L. Gentry, but may be a distinct species. The relatedness of the two taxa is evaluated here through a study of morphology and interfertility toward a decision as to whether or not *J. tlaxcala* should be described as a new species. Although fertile F₁ hybrids have been created, the two taxa are not completely interfertile, and are distinguished by several phenotypic characters.

J. procumbens is a black-fruited perennial herb that is widely distributed in Mexico and Central America, growing in wooded areas, along roadsides, and in disturbed habitats (Gentry and D'Arcy, 1986; Mione, unpublished). *J. tlaxcala* has been found only in central Mexico, and is easily distinguished from *J. procumbens* by its green or light purple fruits. *J. tlaxcala* is considered here as a semi-domesticated or weed species, since it is not known to occur outside of areas of human habitation or agriculture (Mione, unpublished). Although *J. procumbens* and *J. tlaxcala* are not intentionally propagated from seed, each is often tolerated when it arises in cultivated fields, and *J. tlaxcala* is sometimes transplanted into gardens (Mione, unpublished). The fruits of both taxa are consumed by humans, but the sweeter fruits of *J. tlaxcala* are preferred (Williams 1985; Mione, unpublished), suggesting the possibility that

this taxon has undergone artificial selection. Possible modes of selection are speculated on here.

At least twelve characters are described which differ consistently between the two taxa. These include fruit color, fruit size, fruit flavor, calyx shape, calyx color, pedicel shape in cross section, stigma diameter, style width, habit, seed quantity, fruit articulation point, and time of fruit drop. As yet, no naturally occurring plant has been discovered with a morphology intermediate between that of these two taxa. Since a lack of gene exchange may be inferred from consistently distinct sets of characters (De Jong, 1982; Coyne *et al.*, 1988; Grant, 1991), it appears that successful interbreeding between *J. tlaxcala* and *J. procumbens* occurs either rarely or not at all.

Fertile F_1 hybrids have been grown from some of the inter-taxon crosses. These hybrids grew with vigor and produced high percentages of viable pollen along with abundant normal-looking seeds. However, partial intersterility was clearly demonstrated through reduced mean seed size and reduced seed germinability for otherwise viable-looking seeds containing F_1 hybrid embryos. Especially convincing were the tiny malformed seeds that often resulted from inter-taxon crosses, but which were not seen in significant numbers from inter-taxon crosses.

The results of this study are evaluated in the context of the Biological Species Concept (BSC) (Mayr, 1942), whereby species are defined on the basis of gene flow or the potential for gene flow. Genetic barriers to gene flow are believed to result as a byproduct of the speciation process after physical isolation has prevented the

sharing of genetic novelty between diverging populations. As isolated populations diverge, allelic differences accrue, causing reproductive systems to become dissimilar and less compatible (Dobzhansky, 1937; Eldredge, 1985; Mayr, 1988; Coyne *et al.*, 1988; Griffiths *et al.*, 1993).

According to a strict interpretation of the BSC, *J. tlaxcala* should be named only as a subspecies of *J. procumbens*, since the two taxa remain partially interfertile and the speciation process appears to be incomplete. However, species status is recommended here on the basis that a significant genetic discontinuity separates the two taxa, which appear to have experienced significantly different evolutionary histories. By naming *Jaltomata tlaxcala* at the specific rank, we provide ourselves the language with which to describe a genuine evolutionary development within the genus *Jaltomata*.

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INTRODUCTION

The genus *Jaltomata* of the nightshade family (Solanaceae) currently contains various unresolved taxonomic and phylogenetic questions at the species level. A new taxon designated *Jaltomata tlaxcala* (nom. ined.) is closely related to *Jaltomata procumbens* (Cav.) J. L. Gentry, but may be a distinct species. The two taxa are compared here as a supplement to the ongoing research of Thomas Mione (Central Connecticut State University) into the taxonomy and phylogeny of the genus. The relatedness of the two taxa is evaluated through a study of morphology and interfertility, toward a decision as to whether or not *J. tlaxcala* should be described as a new species. Although fertile F₁ hybrids have been created, the two taxa are not completely interfertile, and are distinguished by several divergent characters. Here each taxon will be referred to either as a "species" or as a "race," depending on context.

Jaltomata includes approximately thirty species in two monophyletic subgroups which together range from southeastern Arizona, U.S.A. to southern Bolivia, with one species each occurring on the Galapagos Islands and in the Greater Antilles (Mione and Bye, 1996). The South American lineage contains small woody shrubs with red or orange fruit, while the Mesoamerican lineage is exclusively herbaceous, with fruit commonly purple or black, but sometimes green. *Jaltomata* fruits are small, edible, tomato-like berries with many small seeds (Mione, 1992).

J. procumbens is a black-fruited perennial herb that is widely distributed in Mexico and Central America, growing wild or amongst

human habitation (Gentry and D'Arcy, 1986; Mione, unpublished). Within the overall geographic range of *J. procumbens*, there exist several morphologically distinct races occurring in localized regions. These have been considered as possible variants of the *J. procumbens* lineage, perhaps diverging due to selective forces acting on isolated subpopulations. The large variety of names applied to these races by local people has been cited as evidence of a significant ethnobotanical history (Davis and Bye, 1982; Davis, 1986), and "the occurrence of these populations in disturbed habitats and the encouragement of selected plants in cultivated areas suggest progressive domestication" (Davis and Bye, 1982).

J. tlaxcala is found in central Mexico in the states of Tlaxcala, Morelos, Hidalgo, and Distrito Federal, at altitudes of 2500 - 3000 meters. The fruits of *J. tlaxcala* are consumed by local people, as are those of *J. procumbens*. However, the sweeter fruits of *J. tlaxcala* are preferred when present (Williams 1985; Mione, unpublished). In general, neither species is intentionally propagated from seed, although plants of *J. tlaxcala* are occasionally transplanted into gardens (Mione, unpublished). Each is frequently tolerated when it arises in cultivated fields, and is deliberately not weeded out. Whereas wild *J. procumbens* plants often inhabit wooded areas and roadsides, *J. tlaxcala* is not known to occur outside cultivated sites (Mione, unpublished), and so may be considered a semi-domesticated or weed species.

According to chloroplast DNA characters, *J. tlaxcala* and *J. procumbens* align phylogenetically as sister members of a subgroup of species or subspecies which together have been referred to as the

"*J. procumbens* complex" (Mione, 1992; Mione *et al.*, 1994). Both taxa are diploid, with a chromosome number of $n = 12$ (ruling out divergence by polyploidy) (Mione, unpublished). *J. tlaxcala* has previously been regarded as a morphological variant of *J. procumbens* (Williams, 1985).

The degree of relationship between two related populations may be evaluated in the context of the Biological Species Concept (BSC). As defined by Ernst Mayr (1942), "species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups." The members of a single species may be considered one type of organism by virtue of the fact that they share a cohesive set of genes. Gene flow within a species acts to maintain genetic uniformity.

The BSC has been criticized because it appears to define species solely on the basis of gene flow, even though continuous gene flow cannot always be demonstrated throughout the geographic distribution of a single species (Sokal and Crovello, 1970). The BSC has also been perceived as ignoring the fact that genetic mutations and subsequent selective forces are necessary in order to bring about divergence between two populations; a physical barrier may prevent gene flow, but does not itself bring about a divergent genotype (Ehrlich and Raven, 1969; Levin, 1979). However, it is nevertheless true that gene flow acts against divergence, and that speciation may occur where gene flow between diverging populations is absent or reduced. While it is possible for some members of a species to become isolated from other members of the same species, it is nevertheless true that all members share a

common genetic origin. They must have been united by gene flow at some point in the past, even if only on a local scale. A species is historically an evolutionary unit, whether or not it can be expected to remain genetically cohesive in the future.

Reduced gene flow occurs either where a previously continuous distribution has become separated into patches (perhaps due to physical barriers), or where members of a population have been dispersed into isolated areas (Eldredge, 1985; Mayr, 1988; Grant, 1981, 1991). Gene flow may also be reduced if ecological races of generally sympatric populations are segregated into separate ecological niches (Lewis, 1966; White, 1978; Grant, 1981, 1991). Over time, each isolated subpopulation accumulates genetic novelty (by mutation) which is not shared with the other. The speciation process is enhanced if the isolated populations experience differing selective forces, such that differing mutations are adaptive. Selection is said to be "disruptive" if each of two extreme phenotypes is adaptive but intermediate forms are not (Grant, 1991).

Eventually, the separated populations are likely to differentiate from each other in ways that make them reproductively incompatible, in which case they may not be able to interbreed if brought back together. The biological barriers that arise between divergent populations are both a symptom of divergence and a mechanism for preserving it. Although it is likely that all life on earth is monophyletic (Dobzhansky *et al.*, 1977), genuine biological discontinuities do exist in nature, and "this discontinuity marks the boundary line of the biological species" (or of higher taxa) (Grant, 1991).

Of course, it is entirely possible for human observers to encounter a group of organisms which have proceeded only part way through the speciation process, and which have not diverged in a manner that makes them completely incompatible with their progenitors (Lewis, 1966; White, 1978; Eldredge, 1985; Mayr, 1963, 1988, 1992; Grant, 1981). It is difficult in some cases to know at exactly what point a divergent population has achieved the status of a separate species. Two morphologically divergent populations may be physically isolated, yet may be somewhat interfertile when artificially crossed. Or, two populations may be separated by intersterility, even though they cannot be distinguished morphologically (due to a lack of differing selective forces).

In such cases, natural clues may allow us to judge whether a significant divergence has occurred. A biological discontinuity is indicated if: (1) the two taxa are intersterile, or (2) the two taxa are distinguished by consistent morphological differences. A consistent difference of characters may be considered evidence that interbreeding is not occurring (De Jong, 1982; Coyne *et al.*, 1988; Grant, 1991). It is not always necessary therefore, to prove that two taxa are 100% intersterile before giving them separate specific epithets. On the other hand, subspecific nomenclature may be used if the discontinuity is not substantial enough to insure permanent divergence. When the speciation process is observed at an intermediate point, it is not often possible to know whether divergence will continue in the future.

METHODS

Two approaches were taken in order to evaluate the degree of relationship between *Jaltomata tlaxcala* and *J. procumbens*: (1) Live specimens of each were examined for distinguishing morphological characters. (2) Cross-pollinations were performed within and between each putative species so that inter-taxon fertility could be compared to intra-taxon fertility.

Each putative species was represented by several accessions, each of which is descended either from a single field-collected plant or from a group of field-collected plants in close proximity.

DISTINCT CHARACTERS

Accessions of both putative species were grown in the same two outdoor gardens. Although the growing conditions may have varied slightly between the gardens, both gardens contained examples of each taxon, and all plants within a garden received equal amounts of fertilizer, water, and sunlight. Two separate gardens were used in the hopes of reducing the chance that all plants would be affected by disease. Uniform growing conditions were provided so that environmentally induced morphological plasticity would be less likely to cause differences between the plants. The plants were well spaced out, so that they would not shade each other, or affect each other's growth.

Several characters required no particular measurement, and could readily be observed by sight:

- fruit color at maturity
- calyx color at fruit maturity
- calyx position over fruit
- pedicel shape in cross section
- fruit articulation point
- time of fruit drop relative to fruit maturity
- habit

Fruit color and calyx color were visually surveyed. Since the fruits of *J. tlaycala* fall from the plant at maturity, fruit color was recorded from fruits resting on the ground. Meanwhile, since the time of maturity was judged from fruit color, the time of fruit drop (relative to maturity) was based on color. For example, the fruits of *J. procumbens* do not immediately fall at the time of maturity, and fruit drop may occur as long as four weeks after maximum pigmentation has been achieved. The degree of purple coloration on each accession's calyces was recorded when the associated fruits were mature.

Calyx position was viewed from the side of ripe fruits, at an angle that was perpendicular to the pedicel. No measurements were taken, but it was noted whether or not the calyx hid any part of the fruit from side view, or whether the calyx lobes were held above the fruit, exposing the fruit from side view. The pedicels of each accession were viewed in cross section, since the degree of longitudinal ridging seemed to vary. Some pedicels appeared terete in cross section, while others appeared subterete.

The point where mature fruits disconnect from the plant was observed to occur either where the pedicel meets the peduncle, or where the top of the fruit meets the pedicel. In order to determine

whether this fruit-articulation point is a character distinguishing these two putative species, mature fruits were gently pulled from the plants. However, since this procedure provided very mixed results, the articulation point was instead determined through a search of each accession for empty calyces. On some plants, numerous calyces remained after the fruits had dropped at the end of the growing season, while on other plants the calyces fell with the fruits as the fruits dropped. Fallen fruits sometimes still had calyces attached to them after they were on the ground, indicating an articulation point between the pedicel and the peduncle. However, the absence of a calyx on a fallen fruit could not be considered meaningful, since the calyx may have been lost as the fruit collided with the ground.

Habit also varied between accessions, with some plants being generally procumbent and others being mostly prostrate. A general record was kept of this character, so that trends could be noted. However, the tendency of a plant to grow branches upward and then suddenly fall over due to weight or wind was a complicating factor. Also, some mainly prostrate accessions would occasionally produce an upright branch, usually near the center of the plant.

Other morphological characters were compared quantitatively, and statistical tests were required to evaluate their significance:

- fruit size (length and width)
- seed quantity per fruit
- seed size
- calyx lobe shape (lobe/sinus ratio)
- stigma diameter
- style thickness at midlength

These characters were selected for measurement due to the perception that they varied between the two putative species. Fruit size was measured on ripe fruits only. Each sampled set was chosen randomly, except that each set was made to include two of the largest available fruits (selected specifically) so that the sampled set would reflect an estimate of the maximum possible fruit size. A caliper was used to measure fruit length and width. Since the fruits are sometimes not quite round in cross section, width was measured twice per fruit, in two different directions. These two values were then averaged into one value representing width.

The quantity of seeds per fruit was counted from the same fruits that were used for size measurements. Viable-looking seeds were counted separately from immature and abnormal-looking seeds. The percentage of viable-looking seeds in self-set fruits was recorded for later comparison with the seeds in fruits resulting from inter-taxon crosses.

Seed size was measured and recorded using seeds which had been allowed to dry for at least two months. Due to the fact that the seeds are not symmetrical, it was often difficult to know in which direction to measure length. Also, the thickness of the seeds varied (and may be related to their viability). For these reasons each seed was measured in three directions: length (longest axis), width (approximately perpendicular to the direction chosen as length), and thickness. A value was then calculated for each seed by multiplying length x width x thickness. This value was referred to as "volume," although it does not accurately reflect volume, since

the seeds are rounded. This "volume" value was used for comparison in a statistical test.

The sepals of both species are partially fused, forming calyces with lobes that differ in shape. While the calyx lobes of *J. procumbens* are almost triangular, those of *J. tlaxcala* are more rounded on the sides and more abruptly narrowed at the tips (Figure 1). A sample of calyces was taken from each accession so that lobe lengths and sinus distances could be recorded. The ratio of lobe length to sinus distance served as a quantitative means for describing the difference in shape between the lobes of the two species. Lobe lengths and sinus distances were measured not from the exact center of the calyx, but rather from the edge of the pedicel. One lobe and one sinus distance were measured for each calyx. The sinus chosen for measurement was always immediately next to the measured lobe (Figure 1).

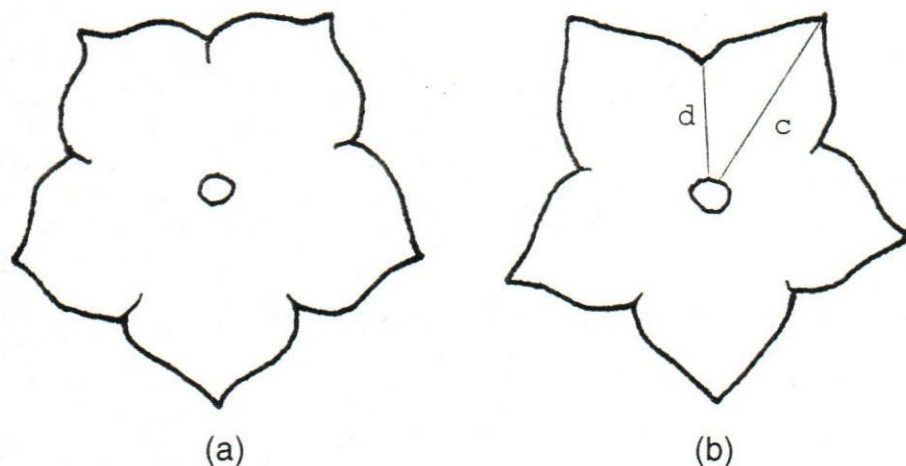


Figure 1. Calyces of *J. tlaxcala* (a) and *J. procumbens* (b). Calyx lobe length (c) and sinus distance (d) are shown.

A membranous area usually occurs just inside the sinus, sometimes with a slit further separating the lobes from each other. This slit was ignored when the sinus distance was measured, and the membranous area was included in the sinus distance. In other words, the sinus distance was measured from the pedicel to where the separation between the lobes began to widen.

At a glance, the flowers of *J. procumbens* and *J. tlaxcala* appear to be almost identical except for the fact that *J. procumbens* more often has pronounced corolla lobules between lobes. However, a visual survey suggested that *J. tlaxcala* has a more abrupt and knob-like stigma, of greater surface area than that of *J. ^{procumbens?} tlaxcala*. Although the actual shape (sometimes knob-like) of the stigma varies considerably even within an accession, stigma diameters were measured on several accessions of each putative species. Since the perimeters of the stigmatic surfaces were often not circular, two perpendicular measurements were made and the average of these two was used as the stigma diameter. Style thickness was also measured, at midlength.

Fruit flavor was the most subjective character studied, since it was judged by humans. However, it was considered worthy of attention, since both Coe and Mione found a noticeable difference between the flavor of *J. tlaxcala* fruits and the fruits of *J. procumbens*. *J. tlaxcala* seems to have a sweet flavor component not present in *J. procumbens*, a flavor which one taster described as a "cheesy blueberry cantaloupe taste" (Halkin, pers. comm.). In fact, fruit flavor may be a very relevant character due to the fact that

J. tlixcala is preferred over *J. procumbens* by local people in Mexico who consume the fruit (Williams, 1985).

A blind taste test was devised in order to determine whether this sweet "cantaloupe" flavor is actually unique to *J. tlixcala* and absent (or less noticeable) in *J. procumbens*. Each taster was given eleven or twelve pairs of fruits and was asked whether the flavors of the two fruits should be called "the same" or "different." Fruits were handed to the tasters one pair at a time, in an opaque jar which prevented the tasters from seeing the fruits. They were instructed not to look at the fruits before eating them. Each pair consisted of one fruit from each of two different accessions. Some pairs contained only one putative species, while other pairs included one example of each putative species. Each taster received almost the same set of assorted pairs that were experienced by the other tasters. However, the order in which these pairs were received was randomized, so that no two tasters received the pairs in the same order. The test was limited to seven tasters due to a shortage of ripe fruits of some accessions.

The taste test was not completely unbiased, since each taster was given two introductory fruits known to represent different flavors. This was done in order to provide the tasters with a knowledge of the degree of flavor variation possible. Without this introduction it is likely that almost all pairs would have been called "different," since fruit flavor varies somewhat between accessions within a putative species, and varies considerably depending on the age of the fruit (even though only ripe fruits were used). It was also necessary to be sure that each taster would not confuse flavor with

other qualities such as seediness or consistency. It was thought that if one of the putative species has a flavor not present in the other, this flavor would be detected by the tasters. Perhaps the fruits of that species would be grouped together in the blind taste test.

INTERFERTILITY

Interfertility between *J. procumbens* and *J. tlaxcala* was tested through a set of manual cross-pollinations between these two putative species. Each putative species was sometimes used as the female parent. Crosses were also made within each putative species (where each parent was a different accession of the same putative species) as a control. A small number of manual self-pollinations were also performed.

Pollinations were planned for the summer of 1995, but could not begin until August due to high temperatures in July which apparently made the pollen non-viable. Since the flowers of both species open after sunrise and close by mid afternoon, most pollinations occurred during morning hours. Also, it was easier to locate virgin flowers in the early part of the day, since bees frequently visited the *Jaltomata* flowers, spreading pollen onto the stigmas. The stigma of each flower used in the manual pollinations was inspected with a 10x or 14x hand lens to insure that no pollen was present beforehand.

On the first day of anthesis, the anthers of both species remain indehiscent (a condition which promotes outcrossing). The filaments of the stamens then elongate on the second day, with the

dehiscent anthers held away from the stigma (Appendix A, Photo 1). On the third day of anthesis the stamens usually bend inward. This brings the anthers close to the stigma and promotes autogamy. Only first-day flowers were used on female parents in manual pollinations. Just prior to pollination, the flower of each female parent was carefully emasculated, so that the flower could not self-pollinate the next day. Pollen was spread evenly on each stigma to be pollinated, from an anther of a selected male parent. Any additional buds or flowers on the inflorescence holding the pollinated flower were removed to avoid confusion in labeling, and to keep the flow of resources to the maturing fruits as equal as possible.

It may be that pollinator-exclusion nets should have been used before and after pollination, to insure that any resulting F_1 plants would truly be the offspring of the intended parental accessions. However, pollinator-exclusion nets were thought to be unnecessary due to the fact that the stigmas were examined for the presence of pollen prior to use, and the raising of hybrids was not expected (because of time constraints).

The plan was to record fruit set, fruit size, and seed quantity, along with seed viability. Provided that each stigma was carefully inspected for stray pollen before manual pollination, and provided that a thick, even layer of selected pollen was applied, it seems unlikely an insect could have added enough additional pollen to significantly affect fruit set or viable seed counts. If *J. tlaxcala* was not compatible with *J. procumbens*, fruit set and seed quantity resulting from these possibly interspecific crosses would perhaps

be very low compared to fruit set and seed quantity resulting from crosses within each putative species. Ultimately however, the study lasted into the summer of 1996, and several hybrids were grown.

As each cross was made, it was assigned a number, and the progress of fruit development was monitored. Since mice and other factors would sometimes cause the loss of a fruit before it reached maturity, the presence and relative size ("large" or "small" compared with others of the same age) of each fruit was recorded at approximately one week and four weeks after pollination. The mature fruits of *J. procumbens* were not removed from the plants until several days after they had reached complete pigmentation. The fruits of *J. tlixcala* were not harvested until they fell naturally from their pedicels. Small pouches constructed of netting were placed around all fruits to prevent their loss in the event of fruit drop.

The success of the crosses made between *J. tlixcala* and *J. procumbens* was evaluated on several criteria:

- fruit set
- fruit size
- seed quantity (viable-looking and non-viable)
- seed size (of otherwise viable-looking seeds)
- % seed germination (of viable-looking seeds)
- vigor and fertility of F₁ hybrids

Of course, some of these criteria are interrelated. For example, fruit size is probably correlated with seed quantity, and seed size may be correlated with seed viability.

If a fruit of significant size was seen to be present at least three weeks following a pollination, that pollination was counted as producing a fruit, even if that fruit was subsequently lost. Fruits were also counted when they reached maturity. Their length and width (width being an average of two measurements per fruit) were measured with a caliper.

Seed quantity was measured by first smashing the fruit on a piece of paper, then counting the seeds that appeared normal and viable. Non-viable seeds were also counted, but were recorded separately. After counting, the seeds were allowed to dry for a period of at least two months before their sizes were measured. Seed size was measured for a random sample from each fruit that resulted from a cross (provided viable-looking seeds had been produced). Only viable-looking seeds were measured for size. Each seed was measured in three directions, and a value was calculated for "volume" as described above.

Seed germination was used to further test seed viability. Only viable-looking seeds were planted, since many could be considered non-viable based merely on appearance (if they were very thin and shriveled, or were white in color). Seeds were carefully counted and individually planted about a millimeter below the surface of carefully moistened potting medium. The potting medium had been autoclaved to reduce the possibility of disease. The seeds were given over two months to germinate, and the percentage that germinated was calculated for each set. Those seeds that germinated but were too weak to push off their seed coats were

nevertheless counted as germinating. A separate record was also kept of these, however.

Note that the germination data was not combined with the viability data that was based on seed appearance. Too many factors affect seed germination. Of course, all seeds had been stored carefully under the same conditions. However, it is not known what storage conditions are most conducive to germination upon planting.

In order to observe hybrid fertility and vigor, fifteen potentially hybrid plants were grown, representing ten different hybrid crosses. For comparison, six plants resulting from intraspecific crosses were also grown, along with one pure accession of each species. These plants were grown during the summer of 1996 in an outdoor garden, where they experienced uniform growing conditions with equal amounts of water, fertilizer, light, and ample weed-free space.

The hybrid plants were examined for the presence of normal-looking seeds and stainable pollen. First however, it was necessary to find morphological evidence that at least some of the plants that were intended to be hybrids were truly a combination of both taxa. This was necessary due to the fact that pollinator exclusion bags had not been used. In order to show that hybridization is possible between *J. tlaxcala* and *J. procumbens*, it was necessary to be sure that the F_1 plants were not merely the result of accidental intraspecific pollination caused by insect pollinators. The hybrids were examined for traits inherited from the male parent which differ from those of the female parent.

Only one putative hybrid had *J. tlixcala* as its female parent, since only one such cross resulted in seeds which germinated. However, this plant showed no characters unique to its male parent, *J. procumbens*. It seems doubtful that this plant was actually the hybrid it was intended to be, since it did not inherit black fruit color, a character thought to be dominant (Mione and Mugaburu, unpublished).

In all other cases, *J. tlixcala* was the male parent, and the hybrid seeds had been harvested from an accession of *J. procumbens* (the female parent). It was therefore necessary to find traits of *J. tlixcala* in the hybrids in order to demonstrate hybridization. Easily distinguished characters were chosen for this purpose:

- fruit color
- fruit size
- maximum seed quantity per fruit
- fruit taste
- calyx color
- calyx position
- pedicel shape in cross section
- fruit articulation point
- plant habit

Most of these characters were evaluated through the same methods as were used for the pure accessions as described above. Also, pressed specimens and photographs were used to record hybrid characteristics. Seed quantity was not measured for a mean, but was measured only for a maximum from the largest fruits. Fruit size was measured from a random sample, except that two of the largest fruits were selected specifically to reflect a maximum fruit

size. No blind taste test was conducted, and taste was evaluated only by Coe (and in some cases Mione). The variability of flavor in the hybrid fruits would probably have made a taste test impractical.

Since the purpose of measuring hybrid characters was to verify that true hybrids had actually been created, it was not considered necessary to evaluate every character on every hybrid. If an intended hybrid clearly exhibited traits of both taxa, it was assumed to be the result of the manually performed cross.

Pollen viability was tested by staining fresh pollen grains in lactophenol cotton blue. One anther per flower was smashed and soaked in stain for 30 minutes. The pollen grains were then examined under magnification. Normal-looking stainable grains were counted separately from abnormal and poorly staining pollen grains. Between 250 and 700 pollen grains were counted for each plant grown.

The percentage of viable pollen was calculated for both hybrid and non-hybrid F_1 plants. Since pollen may be rendered non-viable by disease or by weather, a one-time occurrence of non-viable pollen would not be cause to assume male sterility. It was intended therefore that pollen stainability counts would be repeated, several times if necessary, for any plant with low pollen stainability.

RESULTS

MORPHOLOGY

Clear differences were found between *Jaltomata tlaxcala* and *J. procumbens* for several visually compared characters. Five accessions of *J. tlaxcala* and three accessions of *J. procumbens* were surveyed (Table 1).

TABLE 1. VISUALLY COMPARED CHARACTERS

	<i>J. tlaxcala</i>	<i>J. procumbens</i>
FRUIT COLOR (mature)	green, or with light purple areas	black
CALYX COLOR (mature)	green or yellow	purple, or green with purple
CALYX POSITION	down over fruit, partly hiding fruit in side view, undulating	perpendicular to pedicel, or raised upward, lobes almost flat
PEDICEL SHAPE IN CROSS SECTION	almost terete	subterete, due to longitudinal ridges
FRUIT-ARTICULATION POINT	top of fruit	often between pedicel and peduncle
TIME OF FRUIT DROP	at maturity	up to four weeks after maturity
HABIT	generally prostrate	procumbent

Of five *J. tlaxcala* accessions studied, four displayed mild purple pigmentation at least partially covering their otherwise green fruits (accessions 588, 589, 591, 600). One accession (596) of *J. tlaxcala* never showed any purple color at all, its fruits remaining green, even after they had ripened, dropped, and

subsequently sat on the ground for many days. No accession of *J. tlaxcala* ever showed the intense purple color seen on *J. procumbens*. The pigmentation on the fruits of all three accessions of *J. procumbens* was dark enough to be called "black" (Appendix A, Photos 2, 3).

The fruiting calyces of *J. tlaxcala* were nearly always plain green, or somewhat yellow, with no purple color. Only one accession of *J. tlaxcala* showed any purple at all, and this was so slight as to be nearly imperceptible. All three accessions of *J. procumbens* displayed distinct purple pigmentation blended into the green of their calyx lobes, with some being solidly dark purple (Appendix A, Photos 2, 6).

A visual survey also revealed that the calyces of all accessions of *J. tlaxcala* hang down somewhat over the fruit, as though the fruit is inside a shallow, upside-down bowl (Appendix A, Photo 3). These calyces hide the top of the fruit from side view. Although the calyces on one accession of *J. tlaxcala* hid the fruit only slightly, these calyces nevertheless had the wavy appearance associated with *J. tlaxcala*. The calyx lobes of *J. tlaxcala* have a more pronounced costa, and rise upward in the center, creating an undulating appearance. On all accessions of *J. procumbens*, the calyx lobes were either perpendicular to the central axis of the fruits, or were raised upward and pulled back away from the fruits. Also, the calyx lobes of *J. procumbens* were flatter, with less undulation and a less noticeable costa (Photo 3).

The pedicels of *J. tlaxcala* are closer to terete than are the pedicels of *J. procumbens*, with *J. procumbens* having more distinct

ridges longitudinally. This character is somewhat variable however, and is difficult to distinguish on preserved specimens. The peduncles of both putative species have longitudinal ridges.

The fruits of *J. tlaxcala* have a tendency to drop from the plant at an articulation point located where the pedicel meets the fruit. This was made apparent by the many clusters of empty calyces hanging from all accessions of *J. tlaxcala* in the outdoor gardens at the end of the growing season. Many fruits were scattered on the ground below the plants, but very few had a calyx attached to them. Although it is more difficult to interpret when the fruits of *J. tlaxcala* have reached maturity (since they lack the intense pigmentation of *J. procumbens*), this extra articulation point seems to allow these fruits to drop sooner after maturity than those of *J. procumbens*.

The fruits of *J. procumbens* sometimes remain attached to the plant for up to four weeks after reaching maximum pigmentation. When they finally drop, articulation frequently occurs between the pedicel and peduncle, with the calyx remaining attached to the fruit. Although the fruits occasionally drop by separation from the pedicel, this does not happen often enough to produce the clusters of empty calyces as seen on *J. tlaxcala*.

All accessions of *J. procumbens* grew in an upright habit, the tallest reaching a height of about 1.2 meters. No accession of *J. tlaxcala* ever reached that height, and most plants were generally prostrate. The habit of *J. tlaxcala* is somewhat inconsistent however, and three of the five accessions of *J. tlaxcala* had several

which assumed a more procumbent position. *J. procumbens* may assume a prostrate position when blown over by wind.

Significant differences were also found between the two putative species for several characters which were measured or counted, and then subjected to statistical tests (Table 2). Fruit length, fruit width, and seed quantity all were significantly greater for *J. tlaxcala* when compared with *J. procumbens*. Seed size however, was not significantly different. (Differences are considered significant if the probability is less than 0.05 that the observed results occurred by random chance.)

TABLE 2. STATISTICALLY COMPARED CHARACTERS
(OF FRUITS AND SEEDS)

	<i>J. tlaxcala</i>	<i>J. procumbens</i>	
FRUIT LENGTH (mm)	sample size: 90 range: 7.8 - 14.0 mean: 11.0 std. dev: 1.4	sample size: 81 range: 7.6 - 11.5 mean: 9.7 std. dev: 0.94	P = 8.48×10^{-12} T = -7.3425 d.f. = 169
FRUIT WIDTH (mm)	sample size: 90 range: 9.4 - 17.0 mean: 13.6 std. dev: 1.8	sample size: 81 range: 8.4 - 13.0 mean: 10.9 std. dev: 1.0	P = 0 T = -11.77 d.f. = 169
SEEDS PER FRUIT (quantity)	sample size: 81 range: 36 - 177 mean: 107.28 std. dev: 30.52	sample size: 81 range: 25 - 109 mean: 72.83 std. dev: 20.45	P = 1.6×10^{-13} T = -8.441 d.f. = 160
SEED SIZE (mm ³)	sample size: 149 range: .95 - 2.1 mean: 1.40 std. dev: .21	sample size: 120 range: .51 - 1.9 mean: 1.36 std. dev: .275	P = .0679 T = -1.496 d.f. = 267

(Tests are two-tailed, P = probability, d.f. = degrees of freedom)

The shape of the calyx lobes differs conspicuously between the two taxa; the lobes of *J. procumbens* are more triangular, while the lobes of *J. tlaxcala* narrow at the tips, and are more rounded on the sides (Figure 1; also Appendix A, Photo 6). The ratio of lobe length to sinus distance (lobe/sinus) provided a means for comparing one aspect of the calyx lobe shapes of the two species. A significant difference was discovered, with *J. tlaxcala* having a shallower sinus (and a greater sinus distance) (Table 3).

TABLE 3. STATISTICALLY COMPARED CHARACTERS
(FOR CALYCES AND FLOWERS)

	<i>J. tlaxcala</i>	<i>J. procumbens</i>	
CALYX SHAPE (lobe/sinus)	sample size: 70 range: 1.3 - 2.1 mean: 1.5 std. dev: 0.13	sample size: 56 range: 1.5 - 2.4 mean: 1.8 std. dev: 0.18	P = 1.400×10^{-13} T = 8.277 d.f. = 124
STIGMA DIAMETER (mm)	sample size: 49 range: 0.47 - 1.08 mean: 0.79 std. dev: 0.16	sample size: 36 range: 0.47 - 0.89 mean: 0.60 std. dev: 0.097	P = 9.96×10^{-9} T = -6.371 d.f. = 83
STYLE WIDTH at mid length (mm)	sample size: range: 0.34 - 0.46 mean: 0.40 std. dev: 0.035	sample size: range: 0.24 - 0.39 mean: 0.31 std. dev: 0.031	P = 0 T = -8.8645 d.f. = 42

(Tests are two-tailed, P = probability, d.f. = degrees of freedom)

Stigma diameter and style width are both significantly greater on *J. tlaxcala* than they are on *J. procumbens* (Table 3). The larger stigma of *J. tlaxcala* often has a more abrupt and knob-like

appearance than does the stigma of *J. procumbens*. However, this difference between the taxa cannot be confirmed by sight alone, since the shape and appearance of the stigmas are variable for both species.

The blind taste test resulted in a series of declarations by tasters, stating whether pairs of fruits had approximately the same flavor, or had different flavors. In the event that the two putative species could not be distinguished by fruit flavor, one would expect random results, since the tasters might possibly have based their answers on subtle differences which were due to variable factors such as ripeness. However, the results do not appear to be random.

The seven tasters did not find the three *J. procumbens* accessions to have the same taste as each other. However, of 21 pairs of *J. tlixcala* fruits sampled, 18 pairs were declared to have the same flavor. This high ratio of pairs called "the same" is in contrast with the ratio for the pairs made up of mixed taxa. The fruit flavor of *J. tlixcala* was described as being "different" from that of *J. procumbens* for 31 out of 42 pairs of fruits that contained both of these species.

Table 4 shows all of the fruit pairs as they were administered. Note that this table does not reflect the order in which the pairs were received by the tasters. Although each taster received nearly the same pairs of fruits, the order in which they were received was randomized.

TABLE 4. BLIND TASTE TEST for FRUIT FLAVOR

(D = different flavors, S = same flavor) (accession numbers show pairs of fruits tasted)

taster	BOTH TAXA				<i>J. procumbens</i>		<i>J. flaxcala</i>		
	589	591	600	596	587	586	587	589	596
#1	S	D	D	S	D	D	----	S	S
#2	D	D	D	D	D	D	----	S	S
#3	D	D	D	D	D	D	----	S	S
#4	S	D	S	S	D	----	S	D	S
#5	D	D	D	D	S	S	S	S	S
#6	D	D	S	S	D	----	S	D	D
#7	D	D	D	S	D	----	S	S	S

If *J. tlixcala* has a unique fruit flavor which distinguishes it from *J. procumbens*, we would expect this flavor to be recognized by the tasters, who would then group the *J. tlixcala* fruits together by calling their flavors "the same." The absence of this flavor would also cause the tasters to declare *J. procumbens* fruits "different" from those fruits with the unique flavor.

Notice that taster #5 gave a series of responses matching exactly what we would expect in the event that *J. tlixcala* is distinguishable by taste. Tasters #2 and #3 differed only for two pairs, where both fruits were *J. procumbens*. Results such as these would not be expected to occur by random chance alone. Although the tasters were introduced to the flavor of each accession (with two fruits from each eaten before the test), they could not have grouped the *J. tlixcala* fruits together unless there was a recognizable flavor present.

Also, it is not surprising that pairs of *J. procumbens* fruits would frequently taste different from each other. The fruits of *J. procumbens* used in the taste test had been harvested directly off the plants (not picked up from the ground). However, because the mature fruits can remain on the plant for up to four weeks after reaching maximum fruit color, it is possible that some flavor variation may have been due to differences in the age of the fruits. Since the *J. tlixcala* fruits were picked off the ground (in order to guarantee maturity), they too may have varied in age. Yet their unique flavor was still distinguishable.

INTERFERTILITY

The total fruit-set percentage from interspecific crosses was actually higher than the percentage for crosses made within one species, although the difference was too small to be considered significant. In fact, fruit set was very similar for the two groups of crosses. This is true whether one considers fruit set after three weeks or restricts fruit set to only those fruits that reached maturity. However, it is doubtful that any meaning can be derived from these data. This is emphasized by the fact that seven out of nine manual self-pollinations and intra-accessional cross pollinations failed, even though both species are known to be self-compatible (Davis, 1986; Mione and Bye, 1996; Mione, unpublished). Table 5 summarizes the types of crosses that were made, and shows fruit-set quantities.

It was planned that the fruit sizes and quantities of seeds per fruit resulting from hybrid crosses would be compared to those measurements for non-hybrid crosses. However, there were not enough fruits for a meaningful comparison. These data also cannot be compared to data from self-set fruits, because the flowers producing self-set fruits may have received more pollen. The flowers that produced self-set fruits were not emasculated, and their anthers may have supplied the stigmas with more pollen than would be supplied by a manual pollination.

TABLE 5. FRUIT SET FROM MANUAL CROSSES

CROSSES (female x male)	Quantity of pollinations	Fruit present after 3 weeks	Mature fruits collected
<i>proc.</i> x <i>tlax.</i>	19	13 (68%)	10 (53%)
<i>tlax.</i> x <i>proc.</i>	17	6 (35%)	4 (24%)
TOTAL (interspecific)	36	19 (53%)	14 (39%)
<i>J. procumbens</i>	14	4 (29%)	4 (29%)
<i>J. tlaxcala</i>	13	7 (54%)	5 (38%)
TOTAL (intraspecific)	27	11 (41%)	9 (33%)

A more meaningful comparison can be made regarding seed viability. Self-set fruits and fruits from intraspecific crosses contained seeds which were almost always uniform in size, brown in color, and viable-looking. In many cases however, the interspecific crosses produced odd-looking seeds of various forms, some of which were unusually small, relatively dark, relatively light, or shriveled. In one case a hybrid cross yielded a fruit which had zero normal-looking seeds, but which contained 80 non-viable looking seeds that were tiny and shriveled (Appendix A, Photo 4). Under magnification,

many of these seeds resembled the bran flakes found in breakfast cereal.

The percentage of viable-looking seeds was calculated for hybrid and non-hybrid crosses, as well as for self-set fruits (Table 6). Seed viability (by appearance) was noticeably lower for the hybrid crosses, while the highest seed viability was found for manual crosses within one species. No intraspecific manual cross resulted in a fruit where fewer than 97% of the seeds appeared normal and viable. The self-set fruits had slightly lower percentages (possibly due to non-specific pollen transported by insects). Among the 130 self-set fruits examined, only one fruit had as low as 49% seed viability by appearance.

TABLE 6. SEED VIABILITY (BY APPEARANCE)

Seed source (female x male)	Seeds appearing viable	Seeds appearing non-viable	% appearing viable
<i>proc. x tlax.</i>	412	151	73.2
<i>proc. x proc.</i>	255	1	99.6
Self-set <i>proc.</i>	4,371	146	96.8
<i>tlax. x proc.</i>	61	184	24.9
<i>tlax. x tlax.</i>	414	6	98.6
Self-set <i>tlax.</i>	6,990	407	94.5

A significant size difference was found between otherwise viable-looking seeds from interspecific and intraspecific crosses

(Table 7). The range in seed size was broader for interspecific crosses than it was for non-hybrid crosses, or for any sample group of self-set seeds (Table 2). Also, the average size for seeds containing hybrid embryos was substantially smaller than the average for any group of non-hybrid or self-set seeds. On the other hand, the maximum individual size for a hybrid seed was 2.7 mm³, while the largest seed found in any non-hybrid or self-set sample group was 2.1 mm³. These data (Tables 2, 7) do not include any seeds that were judged to be non-viable based on appearance. Non-viable looking seeds are reported only in Table 6.

TABLE 7. SEED SIZE FROM MANUAL CROSSES
(mm³)

Hybrid	Non-hybrid	
sample size: 90	sample size: 49	P = 1.1 x 10 ⁻⁸ T = 6.081 d.f. = 137
range: 0.48-2.7	range: 1.2-1.9	
mean: 1.1	mean: 1.5	
std. dev: 0.44	std. dev: 0.21	

(Statistical tests are two-tailed, P = probability, d.f. = degrees of freedom)

Seed germination was lower for seeds containing hybrid embryos than it was for seeds containing non-hybrid embryos. The percentage of germination for hybrid seeds was compared to the germination of non-hybrid seeds of the hybrid's maternal parent species (Table 8).

TABLE 8. SEED GERMINATION

Seed source (female x male)	Seeds planted	Seeds germinated	% germinated
<i>proc. x tlax.</i>	342	144	42.1
<i>proc. x proc.</i>	159	118	74.2
self-set <i>proc.</i>	170	124	72.9
<i>tlax. x proc.</i>	58	6	10.3
<i>tlax. x tlax.</i>	217	123	56.7
self-set <i>tlax.</i>	130	47	36.2

In addition to the fact that hybrid seeds germinated in lower percentages, hybrid seedlings (which were counted as germinating) sometimes lacked the vigor necessary to push off their seed coats. Although this only occurred in twenty out of 150 cases, the percentage of weak seedlings was sometimes high within sets of seeds from one fruit. For example, one hybrid cross produced only seven viable-looking seeds. Of these, six germinated, but five were too weak to push off their seed coats. Among the non-hybrid seeds, 100% of the seedlings pushed off their seed coats with vigor.

It should be noted that we know little about the duration of dormancy and the environmental factors which influence the germination of a *Jaltomata* seed. Although the seeds used in the germination test were left in moist soil for over two months, it may be that more would have germinated if left longer. *Jaltomata* volunteers were found germinating in July of 1996 in a corn garden

in Connecticut where experimental *Jaltomata* plants had been grown the year before. These seeds had not only survived winter, but had remained dormant in the soil through the spring, not germinating until summer (Coe, pers. obs.).

All of the putative F_1 hybrids grown in 1996 produced abundant self-set fruits, with nearly all seeds appearing viable. High percentages of viable pollen were found for all hybrids (88-93% stainable), and these percentages were similar to the percentages for non-hybrids (89-96% stainable). The hybrids appeared to be both fertile and vigorous, growing at least as fast and as large as the pure species.

Six of the hybrid crosses resulted in offspring that clearly displayed traits acquired from the intended male parent (*J. tlixcala*), while four did not (and may not actually have been hybrids). All of these six hybrids retained the prominent longitudinal ridges on their pedicels, as well as the black fruit color and purple calyx pigmentation of *J. procumbens*, their female parent. The phenotypic influence of *J. tlixcala* was apparent for these six hybrids however, although the degree of this influence was variable.

For example, the F_1 plants from interspecific cross #49 had a maximum fruit size and a maximum seed quantity (per fruit) which was greater than any recorded for their female parent during this study (Appendix B, Table B-3). They also had the distinctly sweet "cantaloupe-like" flavor of *J. tlixcala*, although the less pleasant "cheesy" taste was not as strong. The calyces were undulated, and usually hid the top of the fruit from side view (Appendix A, Photo 5). Although the lobe/sinus distance ratio was not computed for the

hybrids, the paternal influence on calyx lobe shape was apparent in the rounding of the lobes, which also narrowed at the tips (Photo 6). Fruit articulation on hybrid #49 almost always occurred between the fruit and the pedicel, leaving branches with many empty calyces hanging on them. The plant's habit was distinctly intermediate between that of the procumbent female parent and that of the prostrate male parent.

The presence of so many distinctly paternal characters attests to the fact that the F_1 plants from cross #49 were indeed hybrids, verifying that viable hybrids are possible between the two putative species. Crosses 14, 16, 45, 51, and 52 also yielded convincing hybrids, with many characters appearing intermediate between the two taxa. Although articulation still frequently occurred where the pedicel meets the fruit, the fruit of some hybrids fell only after a greater period of time than is the case with pure *J. tlaxcala*. Fruit flavor for these other hybrids varied, but often seemed to be intermediate between the two species. A detailed description of each hybrid may be found in Appendix B.

Some of the calyces on several hybrid plants were oddly shaped, with extra lobes or malformed lobes. Whether or not this was due to hybridization is uncertain however, since a similar affect was seen on at least one non-hybrid plant, although with much lower frequency. Also, one *J. procumbens* plant grown in 1995 had misshapen calyces, apparently due to disease. However, these calyces did not have extra lobes, and the misshapen lobes on the diseased plant were thinner, rather than wider as on the hybrids grown in 1996. Perhaps the malformed calyces resulted from

conflicting additive alleles, instructing different calyx forms which were not easily combined.

DISCUSSION

At least twelve characters were found which differ consistently between *Jaltomata tlaxcala* and *J. procumbens*, suggesting a significant evolutionary divergence between these two taxa. Since no natural population of plants is known to have a morphology which is intermediate between that of *J. tlaxcala* and *J. procumbens* (Mione, unpublished), this difference does not appear to be due merely to a gradual variation over the range of the *J. procumbens* complex. Instead, the divergent characters appear to represent a well-established biological discontinuity.

The quantity and variety of characters involved indicate that the observed differences are unlikely to be the result of a single gene interaction (epistatic or pleiotropic affect). Although it is possible for one gene to influence the expression of several other genes (Raven *et al.*, 1992; Griffiths *et al.*, 1993), it seems improbable that one gene could control the expression of so many disparate characters. Furthermore, the intermediate morphologies displayed by most of the hybrid plants demonstrate that various alleles act independently to produce the assorted traits. In fact, several individual characters on the hybrid plants often appeared to be influenced by both *J. tlaxcala* and *J. procumbens*.

For example, calyx position and calyx lobe shape resembled *J. tlaxcala* to varying degrees from hybrid to hybrid. On some hybrid plants, this character varied from calyx to calyx, with some calyces appearing more as *J. procumbens*, and others appearing as *J. tlaxcala*. This makes sense only if the calyx position and lobe shape of the

hybrids were influenced by genes from both parents. Perhaps the intermediate hybrid characters were caused by the additive affects of alleles which are usually found in only one taxon or the other. Habit, fruit articulation, and fruit flavor also were found to be phenotypically intermediate on some of the hybrid plants. It does not seem that individual characters such as these could take on intermediate forms without the involvement of multiple divergent alleles.

Artificial selection produces a rapid change in the phenotype of a group of plants, especially for those parts of the plant that are actually used by humans. The cole crops are cited as an example of this: broccoli, cauliflower, cabbage, kale, Brussels sprouts and kohlrabi all appear different from each other, depending on which part of the plant was preferred by humans. Yet these are considered to be varieties of a single species, *Brassica oleracea* (Pickersgill and Heiser, 1976; Rick, 1988). Domesticates usually are not genetically isolated from their wild progenitors, and often may produce fertile hybrids when crossed with them (Harlan, 1983; Heiser, 1990). So, morphological change as guided by humans is not the equivalent of speciation, and may not warrant the use of separate specific nomenclature. Nor does mere ecotypic variation, which can often persist despite significant amounts of gene flow (between interbreeding subpopulations in differing niches) (Begon *et al.*, 1990). A greater discontinuity is required in order for a species boundary to be recognized.

In the case of *J. tlaycala* and *J. procumbens*, a discontinuity is also indicated by partial intersterility. Although the F₁ hybrids

grown in 1996 were vigorous and produced stainable pollen and viable-looking seeds, a degree of sterility was clearly demonstrated through reduced mean seed size and reduced seed germinability for otherwise viable-looking seeds containing F_1 hybrid embryos. Especially convincing were the tiny malformed seeds which sometimes resulted from hybrid crosses (Appendix A, Photo 4). The fact that these non-viable seeds were never observed (in significant numbers) from a non-hybrid cross or self-set fruit demonstrates that this sterility is not a normal occurrence within each putative species (although the *J. procumbens* accessions used all came from central Mexico within or near the range of *J. tlaxcala*, not from the far ends of the *J. procumbens* range, between which, populations might be more divergent from each other). It remains to be seen whether a degree of sterility will be detected in the F_2 hybrid generation. Manual self-pollinations were performed on hybrid #49, so that F_2 plants can be grown in the future.

Note that partial intersterility is not presented here as a *cause* of speciation, since it is not necessarily an affective isolating mechanism. Even if the early generations of offspring from a hybrid cross are partially sterile, recombination in subsequent generations may allow gene segregation for fertility (Schilling and Heiser, 1979). Instead, partial sterility is considered here as a possible *symptom* of divergence.

Barriers due to chromosome rearrangement are possible but unlikely here, since this usually results in very high intersterility (Lewis, 1966; Grant, 1981). This is especially true in diploid hybrids, where an altered chromosome may not be able to pair

properly with its unaltered counterpart (Futuyma, 1979). Correct pairing may occur in polyploid hybrids; however, polyploidy was also ruled out by the fact that both putative species have a chromosome number of $n = 12$ (Mione, 1992).

Reproductive barriers between divergent taxa are believed to arise (in most cases) as incidental byproducts of the speciation process (Dobzhansky, 1937; Grant, 1981; Eldredge, 1985; Mayr, 1988; Coyne *et al.*, 1988; Griffiths *et al.*, 1993). As isolated populations diverge, allelic differences accrue, making reproductive systems dissimilar and less compatible. Ultimately, after a certain degree of genetic differentiation, the populations may achieve complete genetic isolation from each other, prohibiting all chance of hybridization, preventing gene flow, and fulfilling the biological species concept.

Many types of biological barrier may develop between species, acting both before and after pollination. In the case of *J. tlixcala* and *J. procumbens*, lowered hybrid seed viability is caused by an unknown post-fertilization barrier. Other than possible spatial separation (sometimes enforced by humans), pre-fertilization barriers have not been detected thus far. In some groups of plants, pollinator specificity is known to be an isolating mechanism (Linskins, 1983), sometimes even between incipient sibling species (Bateman, 1951). However, the absence of any major difference in floral morphology between *J. tlixcala* and *J. procumbens* suggests that the two probably attract many of the same pollinators (although corolla UV reflectance, nectar chemistry, and chemical attractants were not examined). In the research garden at Central Connecticut

State University, bees appearing to be of the same species were seen visiting accessions of both *J. tlixcala* and *J. procumbens*. Flowers with indehiscent anthers were found to have pollen deposited on their stigmas, showing that pollination by bees is possible. Of course, the observed pollen may have been conspecific, and these pollinator species probably do not occur in the natural habitat of *Jaltomata*.

It is uncertain to what extent humans are responsible for the divergence of *J. tlixcala*. Certainly humans are capable of altering the selective forces acting on a population, through artificial selection and habitat modification. The dual-action of: (1) human selective forces in the domestic environment, plus (2) natural selective forces in the wild environment, might have had a disruptively selective affect, where two extreme phenotypes would be favored over intermediate ones (depending on the ecological circumstances). While certain alleles might be adaptive to an agricultural setting, natural selection would favor the wild-type characters outside of human protection, where domestically-favored characters would probably be much less competitive.

Although humans are not known to deliberately replant selected seeds of either *J. tlixcala* or *J. procumbens*, the perennial nature and long fruiting season of both species provide plenty of time for the recognition of preferred traits and the removal of less desirable phenotypes from cultivated fields. We may speculate that in the past, as desirable alleles accumulated, it became increasingly easy for humans to differentiate between the emerging domestic race and the wild race. Gene introgression between the favored race

and the wild race would be kept low by enforced spatial separation. In addition to this deliberate form of selection, human disturbance seems to have provided a niche which was conducive to the success of alleles ill-adapted to the wild.

Pure-breeding strains are easily isolated for recessive characters in autogamous (or inbreeding) plants such as *J. tlixcala*, because the recessive phenotype is expressed only as a homozygote. The dominant allele is easily detected when present, allowing removal if it is not desired (Pickersgill and Heiser, 1976). It may be speculated that this type of selection allowed the isolation of green (or light purple) fruit color in *J. tlixcala*, since the black color of *J. procumbens* has been shown to result from a dominant allele on a single gene (Mione and Mugaburu, unpublished). It is known that light-colored fruits have often been favored by humans for various domesticated crops (Heiser, 1988; Mione and Bye, 1996). Green fruit color might serve as a tool for preserving the isolation of the domesticated plants. If a green-fruited plant was crossed with regular *J. procumbens*, the presence of wild-type genes would easily be detected in the first generation of hybrids, which would display black-colored fruits.

Admittedly, many of the unique traits of *J. tlixcala* are not recessive, as demonstrated by their expression in the F_1 hybrids. However, it is possible (though currently unknown) that some of the domestically-favored alleles are on genes linked to those for fruit color. Since it is not unusual for adaptive characters to become linked under disruptive selection (Pickersgill and Heiser, 1976), it would be interesting to know if sweet fruit flavor is linked to green

fruit color. When F_2 plants are eventually grown, perhaps linkage will be detected (by the absence of any green fruits without the sweet flavor, assuming sweet flavor is controlled by a single gene).

One can only speculate as to why the divergent characters of *J. tlixcala* might have been selected by humans. The desirability of some qualities such as sweet fruit flavor and larger fruit size are easily understood. On the other hand, it is less evident why the preferred fruit for consumption would be one with more seeds. Perhaps directional selection for larger fruits resulted in an increase in seed quantity because of linked genes or pleiotropy. Since the seeds are small (somewhat smaller than those of the tomato), they may not have been a deterrent to consumption. The larger style width and stigma diameter might correspond with the larger locules needed to hold more seeds. This is reasonable, since the ovary, style, and stigma are all made of the same carpels. An increase in the size of one could very well lead to an increase in the size of the others.

Also as a matter of speculation, the prostrate habit could have been favored by humans, since prostrate plants would appear less to interfere with the more deliberate crops (such as corn) amongst which *J. tlixcala* grows. Meanwhile, a taller plant such as *J. procumbens* is more easily pulled out of the ground by a passing farmer, who does not have to stoop as far in order to get a firm hold on the plant.

The calyx position of *J. tlixcala* (partially hiding the fruit), along with the lack of purple pigment in the calyces and in the fruits, may correspond to a change in dispersal mechanism. Such

changes in fruit dispersal often occur when a race undergoes adaptive radiation into a new niche (Stebbins, 1971). In *J. procumbens* the black fruits are made more visible by the uplifting of the calyx lobes, which are themselves pigmented. Fruit pigmentation is usually considered to be a means of attracting animal dispersers (Raven *et al.*, 1992). However, the mostly-green fruits of *J. tlaxcala* are hidden by their green undulating calyces. The lobes of these calyces are somewhat broader, and the sinus between them is more shallow than that of *J. procumbens*, apparently increasing their tendency to conceal the fruit. These traits may reduce the consumption of fruits by animals.

The plowing and tilling of soil by humans has been suggested as a possible alternative to dispersal by herbivores (Davis and Bye, 1982; Mione, 1996; Hevner, pers. comm.). Seeds would be dispersed only if they escaped consumption by landing out of sight on the ground, where they would later be dispersed during soil preparation. Earlier fruit drop (caused by an extra articulation point) might be favored by this incidental dispersal through human activity.

Dispersal by humans is a trait common in weed species that have become adapted to areas of human disturbance (De Wet and Harlan, 1975). While most domesticates require the deliberate planting of seeds, as well as continued care from humans, *J. tlaxcala* is able to disperse and prosper through its own adaptations. In this regard *J. tlaxcala* is more than a mere domesticate of *J. procumbens*. It is a weed race with its own ecological niche -- the cultivated field -- for which it is specialized.

Niche differentiation may itself act as an isolating mechanism (Mather, 1973; Grant, 1991). The unique alleles that adapt *J. tlaxcala* to its domestic habitat are unlikely to be successful within the wild population of *J. procumbens*, since natural selection probably prohibits an increase in the frequency of such alleles. For example, immediate fruit drop at maturity and green fruit color are not likely to promote dispersal in the wild, and prostrate habit may be of poor competitive value outside the protection of humans. Even if occasional hybridization does occur, a lack of suitable niche space for intermediate forms may cause rapid failure of hybrid combinations. The loss of the human-sponsored niche would presumably mean the end of the *J. tlaxcala* phenotype.

The biological species concept is based on the idea that gene flow occurs within a species, while genetic differentiation prevents gene flow between species. Mayr (1963) has stated that partial sterility between divergent races may be an indication of incipient speciation, and this viewpoint has been shared by many others (*e.g.* Clausen *et al.*, 1947; Stebbins, 1950; Lewis, 1966; Futuyma, 1979; Grant, 1981; Coyne *et al.*, 1988). However, it has also been argued that partially speciated races may not continue to act as cohesive evolutionary units, since they themselves are not necessarily held together by gene flow (Sokal and Crovello, 1970). Gene flow in plants is thought by some to occur very slowly, such that speciation must be a very localized process (Ehrlich and Raven, 1969; Levin, 1993). A condition of relative genetic stasis has been observed to occur in large populations between speciation events (Mayr, 1970, 1992; Gould and Eldredge, 1993). So, *J. tlaxcala* may be the result of

a previous speciation event in a confined area, with little genetic change now occurring.

However, the claim that speciation occurs only on a local scale is itself an admission that gene flow unites an evolutionary unit. If gene flow is not relevant, why must speciation be local? The fact that a newly evolved species disperses (after speciation) does not invalidate the BSC. If some of its subpopulations become isolated and cannot share their novel genes (should any arise) with the other subpopulations, then they too may become divergent new species; and if a new race begins to disperse before complete speciation has occurred, then the completion of speciation will proceed separately in the different isolated subpopulations. Again, these isolated subpopulations might diverge into separate species. Or, as mentioned above, these subpopulations may experience genetic stasis. As explained by Grant (1981), "extensive interbreeding within the population system is not an essential property of biological species; non-interbreeding with other population systems is."

In autogamous species (such as *J. tlaxcala* and *J. procumbens*), gene flow may be slight due to a relatively low rate of outcrossing (Levin, 1971; Schilling and Heiser, 1979). Geographic speciation of widespread populations (as opposed to local speciation) may still be possible in *J. tlaxcala* (and *J. procumbens*) however, since its flowers possess an apparent outcrossing mechanism: the stigma of each flower is receptive for one full day prior to the dehiscence of that flower's anthers. Without an insect pollinator, autogamy may

be delayed until the third day of anthesis, when the stamens bend inward toward the stigma.

Also, gene flow between local populations may be increased by human-sponsored dispersal of fruits. Fruits of *J. tlaxcala* have been seen for sale in outdoor markets in Mexico (Mione, unpublished). They are eagerly eaten, and it is suspected (but not documented) that seeds may germinate after fruit consumption and subsequent defecation of the seeds by humans (Davis and Bye, 1982). It is also possible that seeds are transported within agricultural ecosystems

CONCLUSIONS

A gap of biological discontinuity lies between *Jaltomata tlaxcala* and *J. procumbens*, as demonstrated by character differences and by partial intersterility. However, the creation of fertile hybrids presents difficulties when the relationship of the two taxa is evaluated using the biological species concept. For speciation to be complete according to the BSC, there must be no potential for gene flow. Whereas complete intersterility would preclude any future interspecific hybridization, partial fertility necessitates continued artificial selection or ecological barriers in order to preserve taxonomic distinction. Since *J. procumbens* also occurs in domesticated fields within the range of *J. tlaxcala*, hybridization appears possible. This seems especially true when one considers the high fertility and vigor of the F₁ hybrids grown for this study. Under a strict interpretation of the BSC, *J. tlaxcala* should be named only as a subspecies of *J. procumbens*.

However, the BSC was not designed for the purpose of detecting species boundaries; it was designed to accurately explain the natural reality of the species unit (Grant, 1981; Mayr, 1988, 1992). Although genetic differentiation is a matter of degree, it is nevertheless true that genetic variability is discontinuous, with genetic gaps separating cohesive groups. Morphological distinctions and sterility barriers are clues that two taxa have undergone genetic differentiation, but judgment may still be required in order to evaluate the significance and permanence of this divergence. Genetic barriers are not always complete between closely related

species that are allopatric, or are sympatric but occupy different ecological habitats (Lewis, 1966).

The recognition of *J. tlaxcala* at the specific level may be defended on the basis that *J. tlaxcala* and *J. procumbens* have experienced differing evolutionary and ecological histories. Niche differentiation represents an ecological adaptation that implies more than a mere selection for domestically favored characters. The characters that distinguish *J. tlaxcala* are apparently confined to a specific niche, for which they have evolved. A lack of gene exchange may be inferred from the consistently distinct sets of characters (De Jong, 1982; Coyne *et al.*, 1988; Grant, 1991), and hybrid non-adaptiveness is suggested by a lack of intermediate forms. Partial sterility also is an indication that little or no gene flow is occurring between these taxa, and that genetic differentiation has proceeded beyond the mere selection of domestic traits.

Speciation is a dynamic process which takes place over extended periods of time, so it is not surprising that situations are sometimes found where speciation appears to be incomplete. Yet by naming *J. tlaxcala* at the specific rank, we provide ourselves the language with which to describe a genuine evolutionary development within the genus *Jaltomata*.

APPENDIX A: PHOTOGRAPHS



Photo 1. First-day flower (left) and second-day flower of F_1 hybrid from cross #49, *Jaltomata procumbens* x *Jaltomata tlaxcala*. The anthers of both species remain indehiscent until the second day, promoting outcrossing.



Photo 2. Mature fruits of *J. procumbens* (black) and *J. tlixcala* (mostly green). Note the intense calyx pigmentation possible for *J. procumbens*.



Photo 3. Mature fruits of *J. tlixcala* (left), and *J. procumbens*. Fruits of *J. tlixcala* are green, often with mild pigmentation. Their calyxes hide the top of the fruit from side view, while those of the black-fruited *J. procumbens* do not. Numbered units are centimeters.

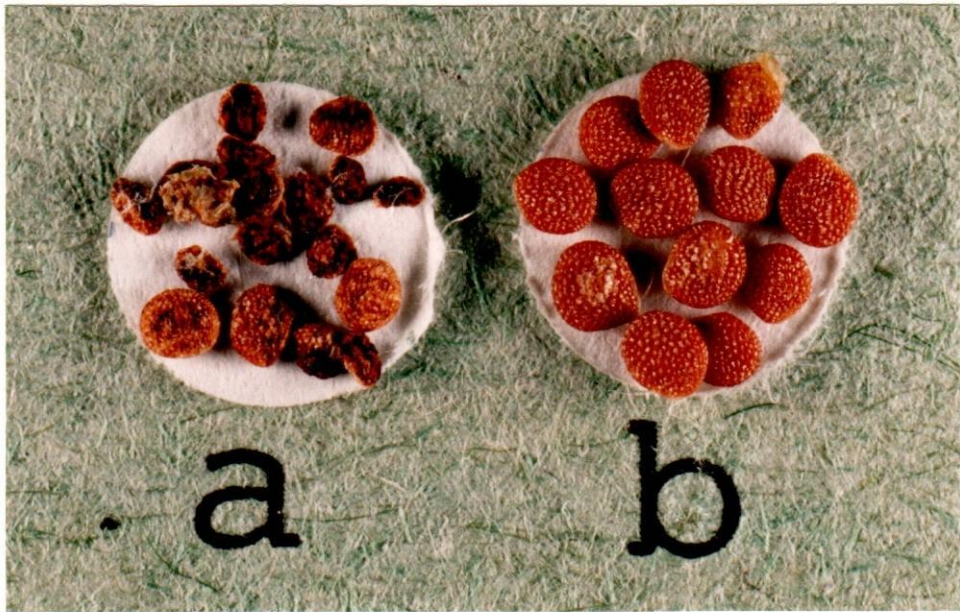


Photo 4. (a) Malformed seeds from a hybrid cross where *J. tlaxcala* was the female parent, pollinated by *J. procumbens*. (b) Normal self-set seeds from the same female parent (about 1.7 mm in length).

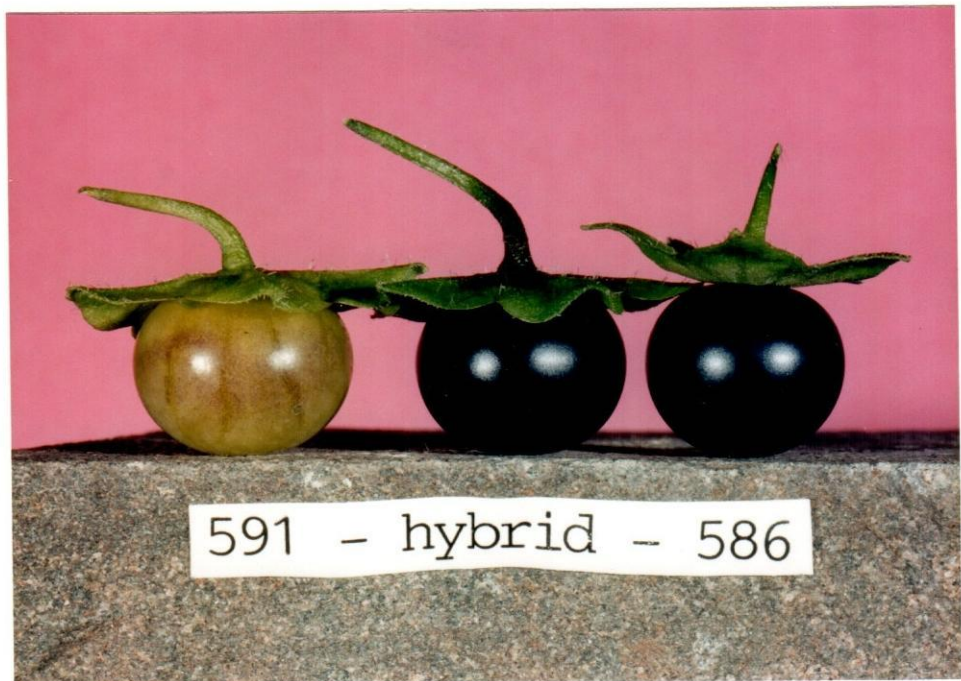


Photo 5. The hybrid fruits from cross #49 (center) had the black pigmentation of their female parent, *J. procumbens* (right). Size and calyx position resembled fruits of the male parent, *J. tlaxcala* (left).

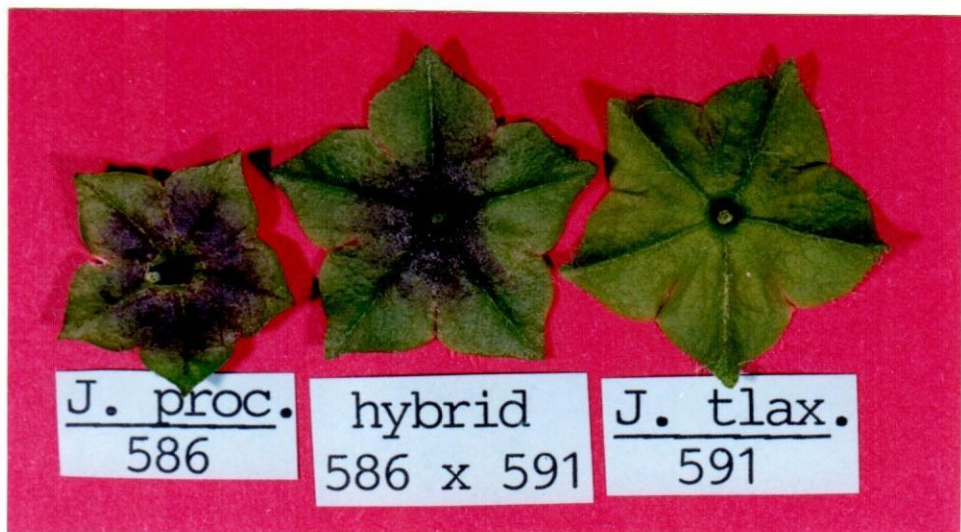


Photo 6. Calyxes of *J. tlixcala*, *J. procumbens*, and of a hybrid made from these parental accessions. Note that the hybrid calyx has the rounded lobes and prominent costa common to *J. tlixcala*, its male parent, while its pigmentation is similar to that of *J. procumbens*, its female parent.

APPENDIX B: F₁ HYBRID CHARACTERS

(for morphologically confirmed hybrids only)

Hybrids from six crosses are described here, in order to demonstrate that hybridization is possible between *Jaltomata tlaxcala* and *Jaltomata procumbens*. Emphasis is placed on characters which were obviously influenced by genes of the male parent (*J. tlaxcala* in all six cases), and which were easily observed. Four other possible hybrids are not described here since they did not show conspicuous paternal traits, and it is not known whether they were truly hybrids or merely the result of unintended intraspecific pollination (by insects) at the time the hybrid crosses were made. The hybrids described here can therefore not be assumed to reflect the range of phenotypes possible in all hybrids, since the four not described here may also be hybrids.

All six morphologically-confirmed hybrids appeared to be vigorous and fertile, producing abundant fruits and large quantities of normal-looking seeds. High percentages of viable pollen (88-93% stainable) were recorded for all flowers examined. All six of these hybrids had black fruits, purple calyx pigmentation and longitudinal pedicel ridges resembling the female parent, *J. procumbens*. Meanwhile, fruit size, fruit flavor, maximum seed quantity, calyx lobe shape, calyx position over the fruit, fruit articulation and habit resembled the male parent (*J. tlaxcala*) to varying degrees between the six hybrids.

Mione's accession numbers are used for differentiating the parent plants (Table B-1). Normal characters (based on 1995 data)

are listed for each pure species for comparison with the hybrids (Table B-2). Following the hybrid descriptions, fruit-size data is also provided for two pure *J. procumbens* F₁ plants from interaccessional non-hybrid crosses, grown in 1996 in the same garden as the hybrids (Table B-4).

Cross #49 produced the most visually convincing hybrid. For this hybrid, fruit length and fruit width are compared to data from one plant representing each parental accession (grown in 1996 under the same conditions as the hybrid) (Table B-3). Mean and maximum fruit width was noticeably greater for this hybrid than it was for any set of fruits from *J. procumbens* (the female parent) grown in 1995 or 1996. Fruit size was more similar to that of *J. tlaxcala* (the male parent). Fruit color, calyx color, calyx position and calyx lobe shape are also compared in photographs (Appendix A, photos 5, 6).

Fruits for measurement were selected randomly in all cases, except that two of the largest available fruits were specifically selected from each plant. This provides an estimate of maximum fruit size.

TABLE B-1. PARENTAL ACCESSION NUMBERS FOR CONFIRMED HYBRIDS

	<i>J. tlaxcala</i> (female parent)	<i>J. procumbens</i> (male parent)
Accessions used:	589, 591, 596, 600	570, 586

TABLE B-2. CHARACTERS OF PURE SPECIES
(1995 data)

	<i>J. tlixcala</i>	<i>J. procumbens</i>
FRUIT COLOR (mature)	green, or with light purple areas	black
FRUIT LENGTH (mm)	sample size: 90 range: 7.8 - 14.0 mean: 11.0 std. dev: 1.4	sample size: 81 range: 7.6 - 11.5 mean: 9.7 std. dev: 0.94
FRUIT WIDTH (mm)	sample size: 90 range: 9.4 - 17.0 mean: 13.6 std. dev: 1.8	sample size: 81 range: 8.4 - 13.0 mean: 10.9 std. dev: 1.0
FRUIT FLAVOR	more sweet, and "cantaloupish"	more sour, not "cantaloupish"
SEEDS PER FRUIT (quantity)	sample size: 81 range: 36 -177 mean: 107.28 std. dev: 30.52	sample size: 81 range: 25 - 109 mean: 72.83 std. dev: 20.45
CALYX COLOR (mature)	green or yellow	purple, or green with purple
CALYX POSITION	down over fruit, partly hiding fruit from side view, undulating	perpendicular to pedicel, or raised upward, lobes almost flat
PEDICEL SHAPE IN CROSS SECTION	almost terete	subterete, due to longitudinal ridges
FRUIT-ARTICULATION POINT	top of fruit	often between pedicel and peduncle
HABIT	generally prostrate	procumbent

F₁ HYBRID CHARACTERS

(F₁ = female *J. procumbens* x male *J. tlaxcala*)

CROSS 14: (female 570 x male 596)

Fruits black at maturity. Mean fruit length 11.6 mm (n = 29), maximum fruit length 13.0 mm. Mean fruit width 14.1 mm (n = 29), maximum fruit width 15.7 mm. Maximum viable-looking seed quantity per fruit 144. Fruit taste variable, but often "cantaloupish." Calyces with purple pigment. Pedicels with noticeable longitudinal ridges. Nearly all fruits articulated where the pedicel meets the fruit. Habit mostly procumbent, but with several prostrate branches. Abundant self-set fruits, many viable-looking seeds, pollen 88% stainable and normal-looking.

CROSS 16: (female 570 x male 600)

Fruits black at maturity. Mean fruit length 10.7 mm (n = 21), maximum fruit length 12.5 mm. Mean fruit width 13.2 mm (n = 21), maximum fruit width 15.1 mm. Maximum viable-looking seed quantity per fruit 156. Fruit flavor only slightly "cantaloupish" if at all. Calyces with purple pigmentation, usually undulating, rarely hanging down and hiding fruit from side view. Pedicels with definite longitudinal ridges. Fruits sometimes (but not always) articulated where the pedicel meets the fruit. Habit procumbent. Abundant self-set fruits, many viable-looking seeds, pollen 92% stainable and normal-looking.

CROSS 45: (female 570 x male 591)

Fruits black at maturity. Mean fruit length 11.6 mm (n = 21), maximum fruit length 13.4 mm. Mean fruit width 13.9 mm (n = 21), maximum fruit width 16.1 mm. Maximum viable-looking seed quantity per fruit 147. Fruit flavor similar to *J. procumbens* but with "cantaloupish" taste also (sort of intermediate between the parents). Calyces with deep purple pigmentation, often solid. Calyces undulating, sometimes hanging down and hiding the fruit from side view. Pedicels with noticeable longitudinal ridges. Fruits sometimes articulated where the pedicel meets the fruit (although most fruits remained on the plant for a long period of time before dropping). Central branches procumbent, but several side branches prostrate and extending as far as five feet away from the center of the plant (these may have been pulled over by weight). This plant was very large and vigorous. Abundant self-set fruits, many viable-looking seeds, pollen 92% stainable and viable-looking.

CROSS 49: (female 586 x male 591)

Fruits black at maturity. Maximum viable-looking seed quantity per fruit 144. Fruit flavor sweet and distinctly "cantaloupish." Calyces with strong purple pigment in the center, green at edges. Calyces undulating and hanging down, hiding top of fruit from side view. Calyx lobes rounded at edges, narrowed at the tips, as on *J. tlaycala*. Pedicels with noticeable longitudinal ridges. Fruits articulated where the pedicel meets the fruit, such that branches are left with many empty calyces hanging. Plant habit somewhat prostrate, but many branches held up off the ground. Habit clearly intermediate between that of parent plants. Pollen 93% stainable and viable-looking.

TABLE B-3. PARENTAL AND HYBRID FRUIT SIZE (for cross 49)
(1996 data)

	Female parent <i>J. procumbens</i> 586	Hybrid 586 x 591	Male parent <i>J. tlaycala</i> 591
FRUIT LENGTH	sample size = 26 range = 9.2-11.9 mean = 10.5	sample size = 34 range = 10.2-13.0 mean = 11.4	sample size = 36 range = 9.3-12.0 mean = 10.8
FRUIT WIDTH	sample size = 26 range = 10.3-13.7 mean = 12.1	sample size = 34 range = 12.2-16.5 mean = 13.9	sample size = 36 range = 11.8-15.6 mean = 13.6

CROSS 51: (female 586 x male 589)

Fruits black at maturity. Mean fruit length 11.4 mm (n = 22), maximum fruit length 12.7 mm. Mean fruit width 13.4 mm (n = 22), maximum fruit width 15.7 mm. Maximum viable-looking seed quantity per fruit 129. Fruits having mild "cantaloupish" flavor. Calyces with purple pigmentation, undulating, and sometimes hanging down over the fruit. Pedicel with longitudinal ridges. Fruits sometimes articulated where the pedicel meets the fruit. Habit procumbent. Abundant self-set fruits, many viable-looking seeds, pollen 93% stainable and normal looking.

CROSS 52: (female 586 x male 589)

Fruits black at maturity. Mean fruit length 11.2 mm (n = 22), maximum fruit length 13.0 mm. Mean fruit width 13.3 mm (n = 22), maximum fruit width 15.6 mm. Maximum viable-looking seed quantity per fruit 131. Fruit flavor often "cantaloupish." Calyces with purple pigmentation. Calyces undulating, often hiding part of the fruit from side view. Pedicels with distinct longitudinal ridges. Fruits sometimes articulated between the fruit and the pedicel. Habit mostly procumbent, but with a few prostrate side branches. Abundant self-set fruit, most seeds viable-looking, pollen 89% stainable and normal-looking.

INTERACCESSIONAL F₁ NON-HYBRID PLANTS

Specimens of pure *J. tlixcala* and pure *J. procumbens* grown in 1996 appeared to have the same characters as those grown in 1995, except that some of the interaccessional F₁ non-hybrid plants were more vigorous, perhaps due only to improved growing conditions. Fruit size was measured for two interaccessional *J. procumbens* plants (Table 4), so that the fruit size of the hybrids could be compared to *J. procumbens* grown under the same conditions as the hybrids. The hybrid fruits (described above) were generally larger, probably due to the male parent, *J. tlixcala*.

TABLE B-4. MATURE FRUIT SIZE OF INTERACCESSIONAL
J. PROCUMBENS
(both crosses: female 586 x male 570)

	Cross #33	Cross #54
LENGTH (mm)	sample size = 22 range = 8.2-11.0 mean = 9.6	sample size = 27 range = 8.7-11.9 mean = 10.6
WIDTH (mm)	sample size = 22 range = 9.7-12.8 mean = 11.2	sample size = 27 range = 10.1-14.3 mean = 12.5

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BIOGRAPHICAL NOTE

I began my formal study of biology at Central Connecticut State University shortly after completing a Bachelor of Arts degree in American Studies at Fairfield University. This radical change of study resulted from a long-standing interest in biology coupled with a desire to expand my education and to obtain an advanced degree.

My career goals remain somewhat vague, but I have many options before me. Certainly I hope for a career in biology, yet I am delighted to have had the opportunity to pursue a subject which interests me, regardless of any professional outcome.

Lawrence A. Coe