ZENTRUM
FÜR BIODIVERSITÄT UND NACHHALTIGE LANDNUTZUNG
SEKTION
BIODIVERSITÄT, ÖKOLOGIE UND NATURSCHUTZ

– CENTRE OF BIODIVERSITY AND SUSTAINABLE LAND USE –
SECTION: BIODIVERSITY, ECOLOGY AND NATURE CONSERVATION

POLLINATION ECOLOGY AND SPATIAL GENETIC POPULATION STRUCTURE OF WILD AND CULTIVATED SPECIES OF CACAO (*THEOBROMA*) IN BOLIVIA

Dissertation zur Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultäten der
Georg-August-Universität Göttingen

im Promotionsprogram Biodiversität und Ökologie
der Georg-August University School of Science (GAUSS)

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Göttingen, August 2013

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Tag der mündlichen Prüfung: 18.09.2013

To my parents,

Betty & Jorge

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SUMMARY

Tropical forest in southwestern Amazonia is threatened by increasing human populations, economic exploitation, deforestation and burning activities for cattle raising and agriculture. Tropical rainforests are characterized by a high level of tree species diversity and consequently low population densities and large distances among conspecifics. The spatial distribution of individuals has substantial consequences for the movement of their pollinators and intraspecific gene flow, hence affecting sexual reproduction. Thus knowledge of gene flow is elementary for understanding the reproductive success and management of tropical tree species. In this study I explored different aspects of the sexual reproduction of wild and cultivated cacao (*Theobroma cacao*) and two wild relatives of cacao (*T. speciosum* and *T. subincanum*) in Bolivia. The hypothesis addressed were: 1) Wild and cultivated plants of *T. cacao* in Bolivia differ genetically; 2) Pollen dispersal distances of wild cacao trees are larger than in cultivated plants; 3) There is some level of gene flow between wild and cultivated plants of *T. cacao*; 4) Pollinator assemblages differ between wild and cultivated populations of *T. cacao* both with respect to taxonomic composition and abundance of floral visitors; 5) Small-scale spatial genetic structure of *Theobroma* species is moderate due to the dependence on animals for both, pollination and seed dispersal. For this purpose, three common species of the genus *Theobroma* of the lowlands of Bolivia were selected, *Theobroma cacao* L. (wild and cultivated), *T. speciosum* Willd. ex Spreng. and *T. subincanum* Mart. These species are cauliflorus understory trees, with numerous small flowers and heavy fruits with fleshy edible seeds. To study the pollen flow of cacao the degree of self-pollination and pollen dispersal distances were estimated as well as gene flow between wild and cultivated cacao. I also explored whether wild and cultivated cacao trees differ genetically. To identify cacao pollinators I documented floral visitor assemblages on wild and cultivated cacao flowers. I further assessed genetic diversity and small-scale spatial genetic structure (SGS) of the three *Theobroma* species and gene dispersal distances were inferred from the patterns of SGS. The results show that wild and cultivated cacao differs genetically, suggesting that “wild” populations of cacao in lowland Bolivia might correspond to truly wild forms or at least to ancient cultivars. Wild populations showed lower genetic diversity than the cultivated cacao, probably because cultivated forms come from a variety of origins (e.g. Costa Rica, Trinidad and Tobago). In contrast to prior studies, I found evidence for self-pollination in both wild and cultivated cacao, even though cross-pollination clearly predominates. Self-pollination events were more frequent in cultivated than in wild cacao. The overall mean pollen dispersal distance was 867 m. Pollen dispersal distances of wild cacao was documented in a range of 10 m to 3007 m, whereas pollen of cultivated cacao was dispersed over distances of 13 m to 2360 m. Pollination distances in cacao were larger than those typically reported in tropical understory tree species, considering the minute sizes of the pollinating midges. The relatively high pollen exchange from cultivated to wild cacao (20%) found, compromises genetic identity of wild populations. Further, a wide range of flower visitors were documented as well as considerable differences between the visitors of wild and cultivated cacao. The findings suggest that midges alone were probably too rare to act as main or even sole pollinators of cacao in our study region. Potential additional pollinators would be small Diptera (e.g., Chloropidae and Phoridae) and Hymenoptera (e.g., Eulophidae and Platygasteridae). The wild relatives of *T. cacao*, *T. speciosum* and *T. subincanum* showed relatively high gene diversity. *Theobroma* species displayed low but significant small-scale spatial genetic structure. *T. speciosum* showed the largest pollen dispersal distance suggesting a more efficient seed and pollen dispersal, whereas *T. subincanum* was the more structured species and had the lowest gene dispersal distance.

In conclusion, improved management of cacao populations should consider the following points: the large pollen dispersal distances reported in cacao and the relatively high pollen exchange from cultivated to wild cacao pointed out the necessity for the protection of extensive natural forests tracts to protect the genetic identity of wild cacao in Bolivia and, in addition, to promote genetic exchange among wild populations to maintain the genetic variability of viable populations. The small-scale spatial genetic structure found in *Theobroma* pointed out the importance of the protection of seed dispersal animals to maintain the genetic resources of this tropical tree genus.

ZUSAMMENFASSUNG

Der tropische Regenwald im südwestlichen Amazonasgebiet ist durch den zunehmenden Bevölkerungsdruck, die wirtschaftliche Ausbeutung, die Abholzung und das Abbrennen großer Flächen für die Viehwirtschaft bedroht. Allgemein zeichnen sich tropische Regenwälder durch eine hohe Diversität von Baumarten und daraus folgenden niedrigen Populationsdichten und entsprechend hohen Distanzen zwischen den Artgenossen aus. Die räumliche Verteilung der Individuen hat grundlegende Konsequenzen auf die Bewegung der Bestäuber und den intraspezifischen Genfluss, was somit die sexuelle Fortpflanzung beeinflusst. Daher ist das Wissen über den Genfluss grundlegend für das Verständnis von Reproduktionserfolgen und dem Management tropischer Baumarten. In dieser Studie untersuchte ich unterschiedliche Aspekte der sexuellen Fortpflanzung von wildem und kultiviertem Kakao (*Theobroma cacao*) und zwei weiteren *Theobroma*-Arten in Bolivien. Folgende Hypothesen wurden angenommen: 1) Wilde und kultivierte Pflanzen von *T. cacao* in Bolivien sind genetisch unterschiedlich; 2) Die Verteilungsdistanz der Pollen wilder Kakaopflanzen ist größer als die der kultivierten Pflanzen; 3) Es existiert ein gewisser Genfluss zwischen den wilden und den kultivierten Pflanzen von *T. cacao;* 4) Die Ansammlung der Bestäuber unterscheidet sich zwischen den Populationen wilder und kultivierter Pflanzen von *T. cacao,* sowohl in Bezug auf die taxonomische Zusammensetzung als auch in der Abundanz der Blütenbesucher; 5) Die kleinräumige genetische Struktur der *Theobroma*-Arten ist aufgrund der Abhängigkeit von Tieren bezüglich Bestäubung und Samenverbreitung gemäßigt. Zu diesem Zweck wurden im Tiefland von Bolivien drei verbreitete Arten der Gattung *Theobroma* ausgewählt: *T. cacao* L. (wild und kultiviert), *T.*

speciosum Willd. ex Spreng und *T. subincanum* Mart. Diese Arten sind stammblütige Bäume des Unterwuchses. Sie haben zahlreiche kleine Blüten und schwere Früchte mit essbaren Samen, welche von Fruchtfleisch umgeben sind. Um den Pollenfluss des Kakaos zu untersuchen, schätzte ich den Grad der Selbstbestäubung und die Distanz der Pollenverbreitung sowie den Genfluss zwischen wildem und kultiviertem Kakao. Ich untersuchte zudem, ob sich die wilden von den kultivierten Kakaobäumen genetisch unterscheiden. Um die Kakaobestäuber zu identifizieren, dokumentierte ich die Zusammensetzung der Blütenbesucher auf wildem und kultiviertem Kakao. Weiterhin ermittelte ich die genetische Diversität und die kleinräumige genetische Struktur (SGS) der drei *Theobroma*-Arten, um die Distanzen der genetischen Verbreitung aus den Mustern der SGS zu schätzen. Die Ergebnisse zeigen, dass wilder und kultivierter Kakao sich genetisch unterscheiden, woraus geschlussfolgert wird, dass die „wilden" Populationen des Kakaos im Tiefland Boliviens tatsächlich wilde Formen oder zumindest sehr alte Sorten darstellen. Wilde Populationen zeigten eine geringere genetische Diversität als kultivierter Kakao, was möglicherweise durch die unterschiedliche Herkunft der kultivierten Sorten (z.B. Costa Rica, Trinidad y Tobago) begründet ist. Im Unterschied zu früheren Studien konnte ich Hinweise für die Selbstbestäubung in wildem und kultiviertem Kakao finden, auch wenn die Fremdbestäubung klar dominiert. Selbstbestäubung war häufiger in kultiviertem als im wilden Kakao festzustellen. Die gesamtdurchschnittliche Distanz der Pollenverbreitung betrug 867 m. Die Distanz der Pollenverbreitung des wilden Kakaos lag in einem Bereich von 10 und 3007 m, während die der Pollen des kultivierten Kakaos über Distanzen von 13 bis 2360 m verbreitet wurden. Die Bestäubungsdistanzen des Kakaos waren höher als die der typischen Unterwuchsbaumarten, wenn man die geringen Größen der bestäubenden Mücken berücksichtigt. Der relativ hohe Pollenaustausch von kultiviertem zu wildem Kakao (20%) gefährdet die genetische Identität der wilden Populationen. Weiterhin wurde eine große

Auswahl an Blütenbesuchern dokumentiert und erhebliche Unterschiede zwischen den Besuchern des wilden und des kultivierten Kakaos festgestellt. Die Ergebnisse lassen vermuten, dass die Mücken als Haupt- oder gar alleinige Bestäuber in unserem Untersuchungsgebiet nicht in Betracht kommen, da sie zu selten vorkommen. Potenzielle zusätzliche Bestäuber sind die kleine Diptera (z.B. Chloropidae und Phoridae) und Hymenoptera (z.B. Eulophidae und Platygastridae). Die wilden Verwandten des *T. cacao*, *T. speciosum* und *T. subincanum* zeigten eine relativ hohe genetische Diversität. Die Arten von *Theobroma* hatten eine niedrige, aber signifikant kleinräumige genetische Struktur. *T. speciosum* zeigte die höchste Distanz der Pollenverbreitung, was auf eine effiziente Samen- und Pollenverbreitung hindeutet, wohingegen *T. subincanum* die strukturiertere Art mit der geringsten Distanz der Genverbreitung war.

Abschließend sollten bei verbessertem Management der Kakao-Populationen folgende Punkte beachtet werden: Die hohe Distanz der Pollenverbreitung bei Kakao und der relativ hohe Pollenaustausch von kultiviertem zu wildem Kakao zeigt die Notwendigkeit des Schutzes weitläufiger Naturwälder. Um die genetische Identität des wilden Kakaos in Bolivien zu schützen und zusätzlich den genetischen Austausch zwischen den wilden Populationen zu unterstützen, sollte die genetische Variabilität lebensfähiger Populationen zu erhalten werden. Die kleinräumige genetische Struktur in *Theobroma* macht die Bedeutung des Schutzes der samenverbreitenden Tiere deutlich, um die genetischen Ressourcen dieser tropischen Baumgattung zu erhalten.

CHAPTER OUTLINE

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This PhD thesis consists of five chapters; chapter 1 includes a general introduction, a description of the study species and introduces the main hypotheses. Chapter 2 on "Gene flow and genetic diversity in cultivated and wild cacao (*Theobroma cacao* L.) in Bolivia", explores the genetic diversity, gene flow and, pollen dispersal distance of wild and cultivated *Theobroma cacao*. In chapter 3 on "Floral visitor diversity and abundance on wild and cultivated cacao (*Theobroma cacao* L.) in Bolivia" explores the floral visitor assemblage on cacao flowers. Chapter 4 on the "Small-scale spatial genetic structure of three wild species of *Theobroma* (Malvaceae)" we assessed small-scale spatial genetic structure (SGS) and genetic diversity of natural populations of *Theobroma* species. Finally, Chapter 5 includes a synthesis of this thesis, addressing the general discussions and the main conclusions.

CHAPTER I

GENERAL INTRODUCTION

A high level of tree species diversity characterizes tropical rainforest. As a consequence, rainforest tree species mostly have low population densities and large distances between conspecifics. The spatial distribution of individuals has substantial consequences for the movement of their pollinators and intraspecific gene flow (Hubbel and Foster, 1983; Ashton, 1984) hence affecting the sexual reproduction. Gene flow has a major influence on the genetic variation within populations, as it balances the detrimental effects of genetic drift, determines effective population sizes, and has important implications for the management and conservation of genetic resources (Fénart *et al*., 2007). As pollen movement is a key component of gene flow, density effects can be assumed to alter genetic structure and, especially in small populations, to increase the probability of extinction (Stacy *et al*., 1996; Ghazoul *et al*., 1998). Considering that tropical forests are experiencing high rates of deforestation (Dick *et al*., 2003), knowledge of gene flow is therefore elementary for understanding the reproductive success and management of tropical tree species.

In contrast to early theories that predicted tropical tree species to be mainly self-fertilizing or inbred (Baker, 1959; Fedorov, 1966), studies of mating systems have revealed that most species are outcrossed and that long-distance pollen dispersal is the norm rather than an exception (Ward *et al*., 2005). Consequently, most studies on the genetic structure of tropical tree species have found high levels of intrapopulation genetic diversity and weak to moderate spatial genetic structuring (e.g. Lacerda *et al*., 2001; Hardesty *et al*., 2005), although exceptions exist (e.g. Degen *et al*., 2004; Dutech *et al*., 2002). In addition, the relative rates of selfing and outcrossing seem to be highly variable both among individuals within populations as well as among populations over years (Ward *et al*., 2005). This might be due to influence of factors such us population density and pollinator abundance (Murawski and Hamrick, 1991; Degen *et al*., 2004), differences in ecological site conditions (Franceschinelli and Bawa, 2000; Ward *et al*., 2005), phenological asynchrony (Murawski and Hamrick, 1992), or degree of disturbance (Dick *et al*., 2003). This appears to be true for both canopy and understory trees (Ward *et al*., 2005).

In the last years much attention has been given to gene flow from crop to wild relatives motivated by concerns about the impacts on wild populations (Ellstrand, 2003; Armstrong *et al*., 2005). Gene flow between crops and wild species can easily occur if they belong to the same species (Ellstrand *et al.* 1999, Snow 2002). Considering the proximity between cacao cultivars and wild cacao populations in Bolivia, exist the possibility of gene flow between both wild and cultivated cacao, currently there is no information on this respect.

Small-scale spatial genetic structure (SGS) is a result of different processes, most importantly as a consequence of limited gene dispersal that causes local pedigrees (Vekemans and Hardy, 2004). As a descriptor of non-random spatial distribution of genetic variation, SGS has important implications for the management and conservation of forest genetic resources, e.g. for sampling designs (Epperson, 1992). Previous studies have found significant effects on SGS of breeding system, life form, seed dispersal and population density (Hamrick and Trapnell, 2011; Zeng *et al*., 2011). Thus – all else being equal – more pronounced SGS is expected in selfing relative to outcrossing species, in small relative to large species, in weakly dispersed relative to well dispersed and in sparse relative to densely growing species. Although there is a tendency of stronger SGS in species with animal vs. wind-dispersed pollen, and in species with gravity vs. animal-dispersed seeds, different types of pollen and seed dispersal did not consistently influence patterns of SGS (Vekemans and Hardy, 2004). Thus, more empirical studies are needed to unravel more general patterns.

The tropical tree genus *Theobroma* L. (Malvaceae) is an excellent study object to enhance our understanding of patterns of reproduction, gene flow and speciation of tropical tree species, as well as being a plant resource of considerable economic interest. We studied three species of the genus *Theobroma*, *T. cacao* L. (wild and cultivated), *T. speciosum* Willd. ex Spreng. and *T. subincanum* Mart. These three species are cauliflorus understory trees, with numerous small flowers, and have heavy fruits with fleshy seeds. The species are pollinated by small insects (Wellensiek, 1932; Soria *et al.*, 1980; Rodrigues and Venturieri, 1997; Azevedo-Rodriguez da Silva and Martins, 2006). Studies on the genetic diversity of *Theobroma* have mostly been restricted to non-natural populations of cultivated *T. cacao* which has been widely distributed (e.g. Lanaud, 1987; Lerceteau *et al.*, 1997) and *T. grandiflorum* (Alves *et al.*, 2007). Cultivars mostly show high levels of genetic variability, although some *Criollo* cultivars have reduced diversity (Lercetau *et al.*, 1997; Motamayor *et al.*, 2002). However, genetic variation is lower in wild populations from southwestern Amazonia – the presumed centre of diversity of *T. cacao* (Silva *et* al., 2011; Thomas *et al.*, 2012) – than in planted populations in lower Amazonia (Sereno *et al.*, 2006). Data on the genetic diversity of *Theobroma speciosum* and *T. subincanum* are lacking.

In *Theobroma cacao*, the distribution of wild populations is uncertain due to millennia of cultivation and local naturalization across the Neotropics (Cuatrecasas, 1964; Chessman, 1944; Warren, 1994). It appears most likely that the species naturally occurs in western or southwestern Amazonia, including northern Bolivia (Whitkus *et al.*, 1998; Motamayor *et al.*, 2003; Sereno *et al.*, 2006). However, it has also been proposed that the “wild” populations of *Theobroma cacao* in Bolivia are the result of naturalized plants from previous cultivation (Tratado de Cooperación Amazónica, 1999).

Relatively little is known about the pollination ecology of *T. cacao*. It is mainly pollinated by tiny midges (Ceratopogonidae) but while knowledge on the sexual reproduction is fairly extensive for cultivated cacao (Wellensiek, 1932; Soria *et al.*, 1980) it is virtually nonexistent for wild populations. *Theobroma cacao* is generally assumed to be an outbreeding species, because of its floral morphology and the occurrence of a self-incompatibility system (e.g. Knight and Rogers, 1955; Falque *et al.*, 1995). However, while self-pollination of a single flower is basically impossible in *Theobroma* due to the flower morphology (Lieberei and Reisdorff, 2007), it has been suggested that geitonogamy might be more common in the wild than assumed (Lanaud *et al.*, 1987). Indeed, the low level of observed heterozygosity in wild populations in upper Amazonia might point to high rates of geitonogamous selfing (Sereno *et al.*, 2006). Most research on *T. cacao* pollination has focused upon the study of midges as the principal pollinating agents (e.g. Soria, 1973; Soria and Wirth, 1974). There is no study on floral visitors of wild cacao populations, is also assumed that wild cacao has the same pollinators as cultivars.

This study explores the reproduction biology of tropical tree species of the genus *Theobroma* (Malvaceae). I studied three understory, shade tolerant, cauliflorous *Theobroma* tree species that are among the most common and widespread species of the genus. In view of the numerous uncertainties surrounding the pollination ecology of *T. cacao*, in this study I set out to document flower visitors in wild and cultivated plants of *T. cacao*. I explored the genetic diversity, the small-scale spatial genetic structure (SGS) and the gene flow of *Theobroma* species in the lowlands of Bolivia.

The main hypotheses addressed in this study are:

1) Wild and cultivated plants of *T. cacao* in Bolivia differ genetically.

2) Pollen dispersal distances of wild cacao trees are larger than in cultivated plants.

3) There is some level of flow between wild and cultivated plants of *T. cacao*.

4) Pollinator assemblages differ between wild and cultivated populations of *T. cacao*, both with respect to taxonomic composition and abundance of floral visitors.

5) Small-scale spatial genetic structure of *Theobroma* species is moderate due to the dependence on animals for both pollination and seed dispersal.

Study organisms

Theobroma cacao L. is widespread in humid lowland forests from southern Mexico to Bolivia and Brazil. Because of its long history of cultivation, the native range of *T. cacao* is uncertain, but most probably located in southwestern Amazonia including Bolivia (Whitkus *et al.*, 1998; Motamayor *et al.*, 2002; Sereno *et al.*, 2006). It has been proposed that the "wild" populations of *T. cacao* in Bolivia are the result of previous cultivation (Tratado de Cooperación Amazónica, 1999). Wild cacao is taller (up to 18 m) than cultivated cacao (to 6 m) (Lieberei and Reisdorff, 2007). Flowers grow in clusters on the tree trunks and branches, are small (corolla diameter 14 mm), and are white with red nectar guides (Cuatrecasas, 1964; Santos *et al.*, 2012). The number of flowers of wild trees is low compared to cultivated varieties. Wild cacao flowers are presumed to be pollinated by tiny midges (Ceratopogonidae, Diptera; Wellensiek, 1932; Soria *et al.*, 1980). The fruits are about 12 cm long, smaller than the cultivated ones, globose to fusiform, with smaller seeds, more pulp, and thicker fruit shells. The seeds are dispersed by gravity, monkeys and sometimes by humans. Squirrels eat ripe pods (Emamdie and Warren, 1993)

Theobroma speciosum Willd. ex Spreng. is widespread in lowland Amazonia below 300 m in Ecuador, Peru, Brazil, and Bolivia. The trees reach 6-18 m in height. The flowers develop in clusters on the trunks, are small (14 mm) and dark red (Cuatrecasas, 1964; Santos *et al.*, 2012). In Brazil, this species is pollinated by various species of drosophilid and phorid flies (Diptera; Azevedo-Rodriguez da Silva and Martins, 2006). The fruits are ellipsoid, segmented (7 segments), 10-12 cm in length and olive green, yellow when ripe (Cuatrecasas, 1964) with 8.1 seeds per pod (Santos *et al.*, 2012). Seeds are 1.8 cm in length (Santos *et al.*, 2012). They are edible, and the local people prepare a sweet drink and chocolate from the seeds. There is some evidence of fruit consumption by monkeys (L.Tube pers. comm.).

Theobroma subincanum Mart. is widespread in lowland Amazonia below 300 m in Colombia, Venezuela, the Guyanas, Surinam, Ecuador, Peru, Brazil, and Bolivia. The trees reach 6-20 m in height. The flowers grow on canopy branches, are slightly larger than those of the other two species (19 mm) and are dark red (Cuatrecasas, 1964; Santos *et al*., 2012). Flowering occurs mainly at the end of the dry season and the beginning of the wet season (September-November). The pollinators of *T. subincanum* are unknown. The fruits are 7-12 cm long, ellipsoidal, brown, and are used to make sweet drinks from the pulp.

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CHAPTER II

GENE FLOW AND GENETIC DIVERSITY IN CULTIVATED AND WILD CACAO (*THEOBROMA CACAO* L.) IN BOLIVIA

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(Published in *American Journal of Botany*)

ABSTRACT

- *Premise of the study*: The role of pollen flow within and between cultivated and wild tropical crop species is little known. To study the pollen flow of cacao we estimated the degree of self-pollination and pollen dispersal distances as well as gene flow between wild and cultivated cacao (*Theobroma cacao* L.).

- *Methods*: We studied pollen flow and genetic diversity of cultivated and wild cacao populations by genotyping 143 wild and 86 cultivated mature plants and 374 seedlings raised from 19 wild and 25 cultivated trees at nine microsatellite loci.

- *Key results*: A PCA distinguished wild and cultivated cacao trees, supporting the notion that Bolivia harbors truly wild cacao populations. Cultivated cacao had a higher level of genetic diversity than wild cacao, presumably reflecting the varied origin of cultivated plants. Both cacao types had high outcrossing rates, but the paternity analysis revealed 7-14% self-pollination in wild and cultivated cacao. Despite the tiny size of the pollinators, pollen was transported distances up to 3 km; wild cacao showed longer distances (mean = 922 m) than cultivated cacao (826 m). Our data revealed that 16-20% of pollination events took place between cultivated and wild populations.

- *Conclusions*: We found evidence of self-pollination in both wild and cultivated cacao. Pollination distances are larger than those typically reported in tropical understory tree species. The relatively high pollen exchange from cultivated to wild cacao compromises genetic identity of wild populations, calling for the protection of extensive natural forests tracts to protect wild cacao in Bolivia.

Key words: microsatellites; paternity analysis; pollen dispersal; selfing.

INTRODUCTION

Gene flow is a major source of genetic variation within populations because it balances the detrimental effects of genetic drift, maintains effective population sizes, and has important implications for the management and conservation of genetic resources (Fénart *et al.*, 2007). In tropical rainforests, where plant species are often characterized by low population densities and large distances between conspecifics, the spatial distribution of individuals has substantial consequences for the movement of their pollinators and intraspecific gene flow (Hubbel and Foster, 1983; Ashton, 1984) leading to, for example, pollen limitation (Murawski and Hamrick, 1991; Ghazoul *et al.*, 1998). Because pollen movement is a key component of gene flow, density effects can be assumed to alter genetic structure and, especially in small populations, to increase the probability of extinction (Stacy *et al.*, 1996; Ghazoul *et al.*, 1998). Considering that tropical forests are experiencing high rates of deforestation, knowledge of gene flow is therefore elementary for understanding the reproductive success and management of tropical tree species.

In contrast to early theories that predicted tropical tree species to be mainly self-fertilizing or inbred (Baker, 1959; Fedorov, 1966), studies of mating systems have revealed that most species are outcrossed and that long-distance pollen dispersal is the norm rather than an exception (Ward *et al.*, 2005). Consequently, most studies on the genetic structure of tropical tree species have found high levels of intrapopulation genetic diversity and weak to moderate spatial genetic structuring (e.g. Lacerda *et al.*, 2001; Hardesty *et al.*, 2005), although exceptions exist (e.g. Degen *et al.*, 2004; Dutech *et al.*, 2002). In addition, the relative rates of selfing and outcrossing seem to be highly variable both among individuals within populations as well as among populations over years (Ward *et al.*, 2005). This might be due to the influence of factors such as population density and pollinator abundance (Murawski and Hamrick, 1991; Degen *et al.*, 2004), differences in ecological site conditions (Franceschinelli and Bawa, 2000; Ward *et al.*; 2005), phenological asynchrony (Murawski and Hamrick, 1992), or degree of disturbance (Dick *et al.*, 2003). This appears to be true for both canopy and understory trees (Ward *et al.*, 2005).

The tropical tree genus *Theobroma* L. (Malvaceae) is an excellent study object to enhance our understanding of patterns of reproduction, gene flow and speciation of tropical tree species, as well as being a plant resource of considerable economic interest. Although several of the 20 *Theobroma* species in tropical America are locally harvested, the cultivars of *Theobroma cacao* L. have by far the main commercial importance. Surprisingly little is known about the pollination ecology of *T. cacao*. It is mainly pollinated by tiny midges (Ceratopogonidae) but although knowledge on the sexual reproduction is fairly extensive for cultivated cacao (Wellensiek, 1932; Soria *et al.*, 1980) it is virtually nonexistent for wild populations. *Theobroma cacao* is generally assumed to be an outbreeding species, because of its floral morphology and the occurrence of a self-incompatibility system (e.g. Knight and Rogers, 1955; Falque *et al.*, 1995). However, although self-pollination of a single flower is basically impossible in *Theobroma* due to the flower morphology (Lieberei and Reisdorff, 2007), it has been suggested that geitonogamy may be more common in the wild than has been assumed (Lanaud *et al.*, 1987). Indeed, the low level of observed heterozygosity in wild populations in upper Amazonia might point to high rates of geitonogamous selfing (Sereno *et al.*, 2006).

Lately, much attention has been given to gene flow from crop to wild relatives, motivated by concerns about the impacts on wild populations (Ellstrand, 2003; Armstrong *et al.*, 2005). For gene flow between crops and wild relatives to occur, several conditions have to be fulfilled: both types must co-occur within the pollen or seed dispersal range, the flowering season must overlap, hybrid offspring must be viable and fertile, and the establishment of genes in the crop or the wild relative must take place (Ellstrand, 2003). Gene flow between crop and its wild relative can easily occur if they belong to the same species (Ellstrand *et al.*, 1999; Snow, 2002). This phenomenon exists since the origin of the agricultural domestication where crop-wild hybrids are often viable and fertile (Hauser *et al.*, 1998, Snow *et al.*, 1998). Crop-to-wild gene flow has been observed in many species, including fruit trees, e.g. in the genus *Prunus*, in which bidirectional gene flow between the cultivated almond tree (*Prunus dulcis*) and its wild counterparts (*Prunus orientalis*) has been reported (Delplancke *et al.*, 2011). Considering the possible impacts on wild populations, the wild progenitor normally has higher genetic diversity, and this reservoir of genetic diversity could be threatened if swamped by depauperate domesticated types. As a consequence extinction by displacement of native allelic diversity may occur, as has been observed in the wild walnut (*Juglans californica* Wats. var. *californica*) and in Catalina mountain mahogany (*Cercocarpus traskiae* Eastw.; Rieseberg and Gerber, 1995). Similarly, as a result of human cultivation, only a few truly wild populations of coconut (*Cocos nucifera* L.), date palm (*Phoenix dactylifera* L.), and the olive (*Olea europea* L.) remain (*e.g.*, olive: Bronzini de Caraffa *et al.,* 2002). However, crop-wild gene flow could also be considered positive if disease or pest resistance genes were transferred to wild populations. Consequently, the ecological impacts in natural ecosystems and the long-term effects of the gene flow between crops and their wild relatives require more research (Wolfenbarger and Phifer, 2000; Ellstrand, 2003).

In *T. cacao*, the distribution of wild populations is uncertain after millennia of cultivation and local naturalization across the Neotropics (Cuatrecasas, 1964; Chessman, 1944; Warren, 1994). It appears most likely that the species naturally occurs in western or southwestern Amazonia, including northern Bolivia (Whitkus *et al.*, 1998; Motamayor *et al.*, 2003; Sereno *et al.*, 2006). However, it has also been proposed that the “wild” populations of *T. cacao* in Bolivia are the result of naturalized plants from previous cultivation (Tratado de Cooperación Amazónica, 1999). In Bolivia, cacao was first cultivated ≥200 yr ago by the native Moseten ethnic group who, encouraged by missions and Bolivian government (Villegas, Mayor de San Andrés University, unpublished), grew clones from Ecuador, Trinidad and Tobago and Costa Rica (Somarriba and Trujillo, 2005). Yet, data on the genetic diversity of both wild and cultivated cacao types are lacking from Bolivia.

Our study took place in the northeast lowlands of Bolivia where presumed wild forms of cacao occur patchily in the natural forest. In addition, farmers cultivate commercial hybrids of both the *Forastero* and *Criollo* cultivars provided by nongovernmental organizations. The objectives of our study were: 1) to establish whether wild and cultivated cacao differ genetically; 2) to quantify self and cross-pollination; 3) to estimate pollen dispersal distance of wild and cultivated cacao trees; and 4) to test whether gene flow occurs between wild and cultivated cacao.

We hypothesized that wild and cultivated cacao plants in Bolivia differ genetically. On the other hand, we hypothesized that pollen cacao dispersal distance of wild cacao trees is larger than that of cultivated plants and that gene flow occurs between the two cacao types occur.

MATERIALS AND METHODS

Study species—*Theobroma* includes about 20 species of cauliflorous trees native to tropical America (Cuatrecasas, 1964; Rondón and Cumana-Campos, 2005), most of them understory, shade tolerant species. *Theobroma* is famed as the source of cocoa, chocolate and vegetable butter, and has been used since antiquity (Wood and Lass, 1985). Although several species are locally harvested, the cultivars of *T. cacao* L. are by far the main commercial taxa, representing the world's second most important tropical cash crop species, supporting a $5 billion industry and 40-50 millions of farmers (FAO statistical databases: http://faostat.fao.org). These cultivars involve partly polyembryonous forms as well as crosses with *T. bicolor* Bonpl., *T. angustifolium* D.C., and *T. grandiflorum* (G. Don f.) Schumann (Addison and Tavares, 1952; Cuatrecasas, 1964; Martinson, 1966). Wild cacao has been described as growing taller (to 18 m) than cultivated cacao (to 6 m) (Lieberei and Reisdorff, 2007), but given the difficulty of discerning truly wild populations and different growth and management conditions in plantations and forests, this difference may not be meaningful. Wild cacao further tends to have smaller fruits with smaller seeds, more pulp, and thicker fruit shells.

Flowers are white with red nectar guides, with 5 petals ~10 mm long, and grow in clusters on the tree trunks. The fruits are ellipsoid to elongated; segmented (10 segments); 15-19 cm in length; greenish, orange or red when ripe; and dispersed by gravity and mammals. Anthesis occurs at dawn, and anther dehiscence takes place throughout the morning hours (Young *et al*., 1987). Each flower produces ~14000 pollen grains (Massaux *et al*,. 1976). Flowering phenology of *T. cacao* is seasonal, varying between genotypes and in response to local climatic conditions, but usually being highest at the onset of the rainy season (Paulin *et al*., 1983). In Bolivia, the main flowering season is in September-October for wild trees and October-November for the cultivated ones, although some flowering occurs throughout the year among the cultivated trees (C. Chumacero, unpublished data). The flowers are receptive for 1 day, and unpollinated flowers drop after ~2 days. *Theobroma cacao* is pollinated by insects and has no wind pollination (Harland, 1925). Ceratopogonid midges are considered to be the principal and most effective pollinators of cacao, at least in cultivars (e.g. Billes, 1941; Posnette, 1950; Brew and Borman, 1993). Currently it is unknown how far these tiny midges transport the pollen grains and whether they accomplish gene flow between wild and cultivated cacao. Many authors have reported self- incompatibility in cacao (e.g. Pandey, 1960, Falque *et al.*, 1995), yet the incompatibility system in cacao is not absolute, but quantitative, depending on the ratio of fertilized and non-fertilized ovules. Furthermore, self-compatible genotypes exist (e.g., the commercially used ICS 1 cultivar; Smulders *et al.*, 2008).

Study sites and sampling—We studied cultivated and wild *T. cacao* trees on the lands of the native Tacana community Napashi (67°52’17.4’’ S -14°5’20.6’’ W) in the northeast lowlands of Bolivia. Here, presumed wild forms of cacao occur patchily in the natural forest while farmers cultivate commercial hybrids of both the *Forastero* and *Criollo* cultivars provided by nongovernmental organizations. The farmers commonly germinate seeds from wild trees and later graft the cultivar clones on these. For our study, we selected five cultivated plantations along a 6-km path leading into the forest and identified all wild trees in the forest up to 400 m away from the path (Fig. 2.1). Further exploration of the area was inhibited by the inaccessibility of the area. Mean (± SD) nearest neighbor distances were 8.0±5.9 m and 63.6±224.1 m for cultivated and wild trees, respectively.

We sampled leaves from both parental trees and offspring. Altogether, 603 samples of fresh leaf material were collected in 2009 and 2010. Of these, 229 samples corresponded to parental trees (86 from cultivated and 143 from wild trees), from 44 of which seeds were collected from ripe pods and from which 374 seedlings were raised (185 seeds from 25 cultivated and 189 from 19 wild trees).

Microsatellite analysis—DNA extraction was performed with a standard protocol adapted from Doyle and Doyle (1987) using 20-mg silica-gel-dried leaf material and a modified extraction buffer [2% alkyltrimethylammoniumbromide, 0.1 M TRIS–HCl, 0.02 M disodium-EDTA (pH 8.0), 1.4 M NaCl, 1% polyvinylpyrrolidone]. Samples were genotyped at nine microsatellite loci previously established by Lanaud *et al.* (1999) and Saunders *et al.* (2004) in one simple and four duplex reactions: mTcCir6 + mTcCir25, mTcCir11+ mTcCir 12, mTcCir15 + mTcCir21 (annealing temperature 46°C), mTcCir7 + mTcCir18 and mTcCir1 (51°C). Polymerase chain reaction (PCR) assays were set up in final volumes of 25 µl, containing 20 ng/µl of genomic DNA, 0.8/0.5 µl of primer (5 pmol/mL; Metabion International AG, Martinsried, Germany) for the locus with longer/shorter fragment length, 2.5 µL dNTPs (2 mM; Q-Biogene, Montreal, Quebec, Canada), 0.2 µl Taq DNA polymerase (5U / µl, MP Biomedicals, Solon, Ohio, USA), 2.5 µl incubation mix T. Pol with 1.5 mM MgCl2 (MP Biomedicals) and 16.8 µl H2Obidest. PCR was performed in a Mastercycler gradient or Mastercycler epgradient (Eppendorf, Hamburg, Germany) under the

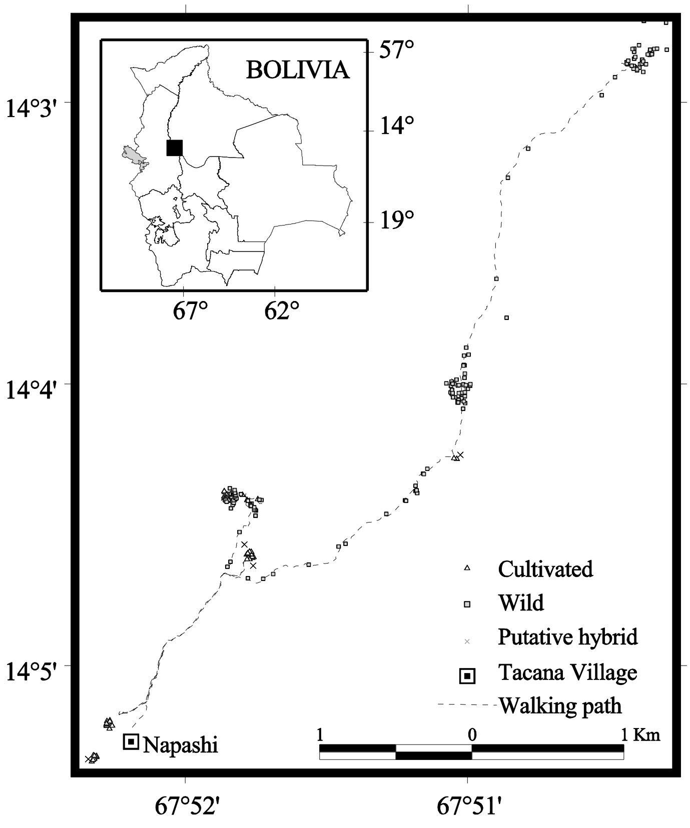

Fig. 2.1 Location map of the study area and the sampled trees.

following temperature regime: 94°C for 4 min, 32 cycles with 30 s at 94°C, 60 s at 46°C or 51°C, and 60 s at 72°C, and a final 3 min at 72°C. 2 µl PCR products (1:5 diluted) were used for separation on a MegaBace sequencer 1000 system (Amersham Bioscience, Uppsala, Sweden) with MegaBace-ET Rox 400 (Amersham Bioscience) as a size standard. The genotyping was performed with the MegaBace Fragment Profiler software 1.2 (Amersham Bioscience)

Data analysis— To explore overall genetic structure of wild and cultivated trees and to identify putative hybrids, we first performed a principal component analysis (PCA) calculated with GenAlEx 6 (Peakall and Smouse, 2006). Second, we applied a Bayesian cluster approach, using Structure version 2.3.3 (Falush *et al*., 2007) which groups individuals into clusters representing homogeneous gene pools without a priori information about individual origin. We ran 10 replicate runs, with the number of clusters (*K*) ranging from 1 to 10, of an admixture model with correlated allele frequencies, with 25 000 burn-in and 50 000 subsequent Markov-Chain-Monte-Carlo repeats. The most probable *K* value was determined following Evanno *et al*. (2005). Replicate runs were averaged using Structure-sum (Ehrich, 2011). We assessed genetic variation for spatial groups of trees and estimated gene diversity (H_E), number of alleles (A) and allelic richness (AR), which is a measure of allelic variation correcting for difference of sample size using Fstat version 2.9.3.2. Diversity levels were compared between wild and cultivated cacao by resampling 1000 times in Fstat (Goudet, 2001).

To study the pollen flow of cacao we performed a paternity analysis using Cervus version 3.0.3 (Kalinowski *et al*., 2007). We used multilocus genotypes of adult plants (maternal trees and candidate fathers) and seedlings of known mothers (see Appendix 2.4, 2.5). Cervus identifies the pollen donors of each seedling so that pollen dispersal distance can be deduced. The levels of gene diversity of *T. cacao* varied between 0.3 and 0.6 among loci, indicating adequate variability for pollen dispersal estimation (Ritland, 2002). To reduce the error in the paternity assignment, the samples with ≥3 mismatches with the maternal tree genotype were excluded (n=32). Further, null allele frequency was estimated with Cervus. The primers had null allele frequencies ≤0.2 (Appendix 2.1). The most likely parents were determined using the Delta statistic (Marshall *et al*., 1998) using the allele frequency of the studied individuals as a reference. The significant critical Delta value to assign paternity was calculated through paternity simulations in Cervus. The difference between the individual with the highest Delta value and the second-most-likely candidate's should be greater than the critical Delta value to accept a paternity. For the paternity simulation, we used the following parameters: number of offspring: 10 000; number of candidate fathers: 100; proportion of candidate fathers sampled: 0.30. To test auto-fertilization, known mothers were tested as candidate fathers. Proportion of loci typed: 0.993; proportion of loci mistyped: 0; minimum number of typed loci: 5. We considered a null scoring error (proportion of loci mistype) taking in account the study of Oddou-Muratorio *et al*. (2003) who pointed out that even when scoring errors occurred at a high rate, it was better to assume a null level scoring error in Cervus in order to avoid the increase of type I error (false-positive paternity).

G-tests were applied to compare self- and cross-pollination in wild and cultivated cacao trees. To test for differences in distributions of pollen dispersal distance between wild and cultivated cacao, we applied a *Kolmogorov-Smirnov* test.

RESULTS

The PCA distinguished two main groups of *T. cacao* individuals, which correspond to cultivated and wild plants. Ten individuals were placed between these two main groups and presumably corresponded to hybrids between cultivated and wild cacao (Fig. 2.2). Bayesian cluster analysis with Structure fully matched the PCA analysis and revealed two clusters, representing wild and cultivated plants, respectively (Fig. 2.3; and see Appendix 2.6). Two individuals turned out to be a wild genotype in a cultivated field and a cultivated genotype in a wild population, respectively. In two cultivated fields, seven individuals (i.e., 9.6% of all cultivated plants) had admixed genotypes with nearly equal contribution of wild and cultivated gene pools, suggestive of the presence of first generation hybrids between wild and cultivated genotypes (Fig. 2.3). Cultivated cacao had a higher level of gene diversity than wild cacao, with mean (± SD) H_E values of 0.673 ± 0.055 and 0.391 ± 0.043), respectively ($P = 0.001$). The cultivars also showed higher allelic richness (AR = 4.84) and higher number of alleles (A = 43) than wild cacao (AR = 2.91; A = 26; $P = 0.001$; Table 2.1).

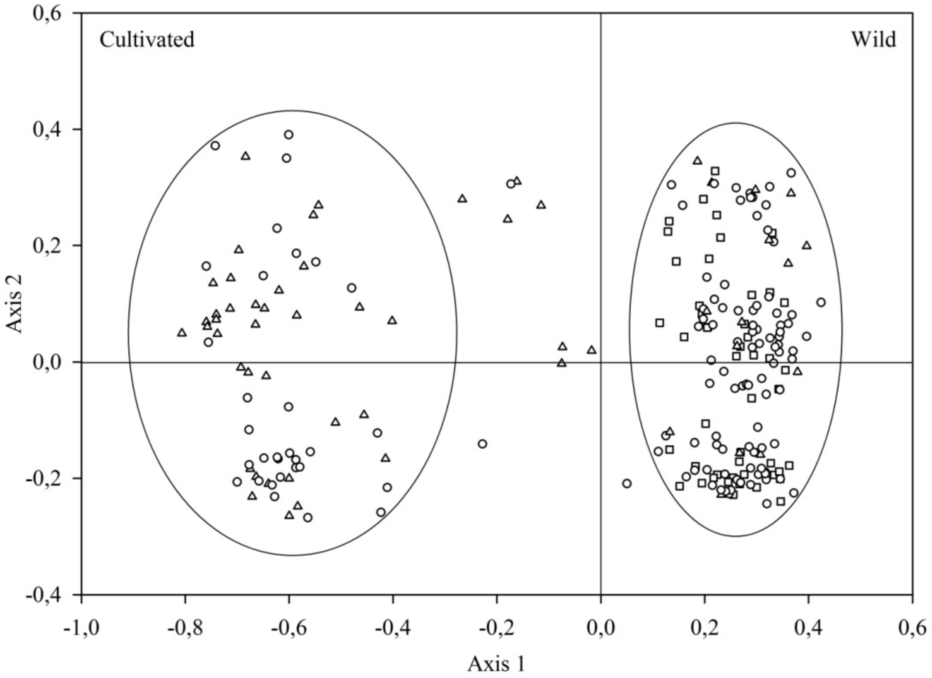

Fig. 2.2 Principal component analysis of microsatellite data for 229 adult trees of *Theobroma cacao* (explained variance: axis 1: 54%, axis 2: 12%), including wild cacao trees (squares), cultivated cacao trees (triangles); and unknown trees (circles).

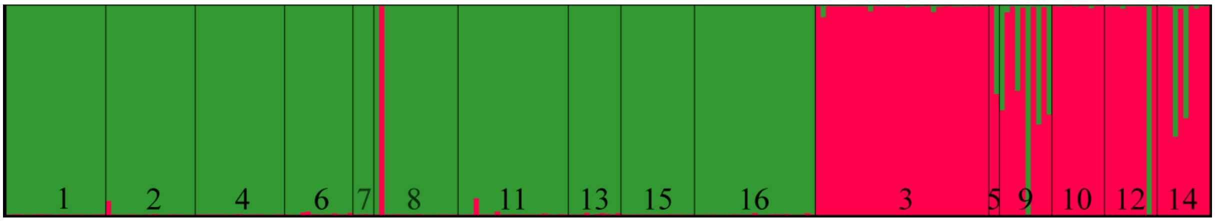

Fig. 2.3 Mean results of 10 runs of Structure at *K* = 2 for 229 *Theobroma cacao* individuals in 16 wild or cultivated groups. Note that one individual in group 9 (↓) was known to be wild.

Parentage analysis revealed that self-pollination occurred in 7% of wild and 14% of crop seedlings of *T. cacao*. Accordingly, 86–93% of all seedlings were considered the result of cross-pollination (Table 2.2). Self-pollination events were significantly more frequent in cultivated than in wild cacao (*G*-Test, *G* = 2.89, df = 1, *P* = 0.044).

TABLE 2.1. Genetic diversity of wild and cultivated *Theobroma cacao.* Allelic richness (AR) was calculated with a sample size of 220 individuals. *n* = sample size, H_E = expected heerozygosity, and A = number of alleles.

Group	Cacao type	*n*	H_E	A	AR
G01	Wild	19	0.372	24	2.67
G02	Wild	17	0.463	27	2.98
G04	Wild	14	0.374	25	2.78
G06	Wild	13	0.407	26	2.83
G08	Wild	16	0.331	27	3.00
G11	Wild	21	0.396	30	3.33
G13	Wild	11	0.415	26	2.89
G15	Wild	14	0.322	23	2.56
G16	Wild	23	0.371	29	3.22
Mean	**Wild**	**16**	**0.38**	**26**	**2.92**
G03	Cultivated	33	0.653	46	5.10
G09	Cultivated	10	0.756	51	5.67
G10	Cultivated	10	0.635	33	3.67
G12	Cultivated	9	0.627	38	4.22
G14	Cultivated	10	0.709	50	5.56
Mean	**Cultivated**	**14**	**0.676**	**44**	**4.84**

Paternity could be assigned to 17% of the studied seedlings, considering a relaxed confidence level (Appendix 2.2). We found that 11 out of 99 alleles were private to the offspring (Appendix 2.3), indicating that the overall low rate of paternity assignment was partly due to pollen immigration. The overall mean (± SD) pollen dispersal distance was 867 ± 888 m. Pollen dispersal distance of wild cacao was observed in a range of 10 m to 3007 m (mean = 922±1030 m), whereas pollen of cultivated cacao was dispersed over distances of 13 to 2360 m (mean = 826±804 m; Fig. 2.4). Pollen dispersal distances were significantly larger in wild than in cultivated cacao (Kolmogorov-Smirnov test, KS = 1.323, df = 1, $P = 0.03$).

TABLE 2.2. Paternity assignment of 342 seeds of wild and cultivated *Theobroma cacao*

Paternity	**Cultivated**		**Wild**	
	n	**%**	**n**	**%**
Assigned: self-pollination	21	14%	13	7%
Assigned: cross-pollination	13	8%	10	5%
Unassigned	121	78%	164	88%
Total	155	100%	187	100%

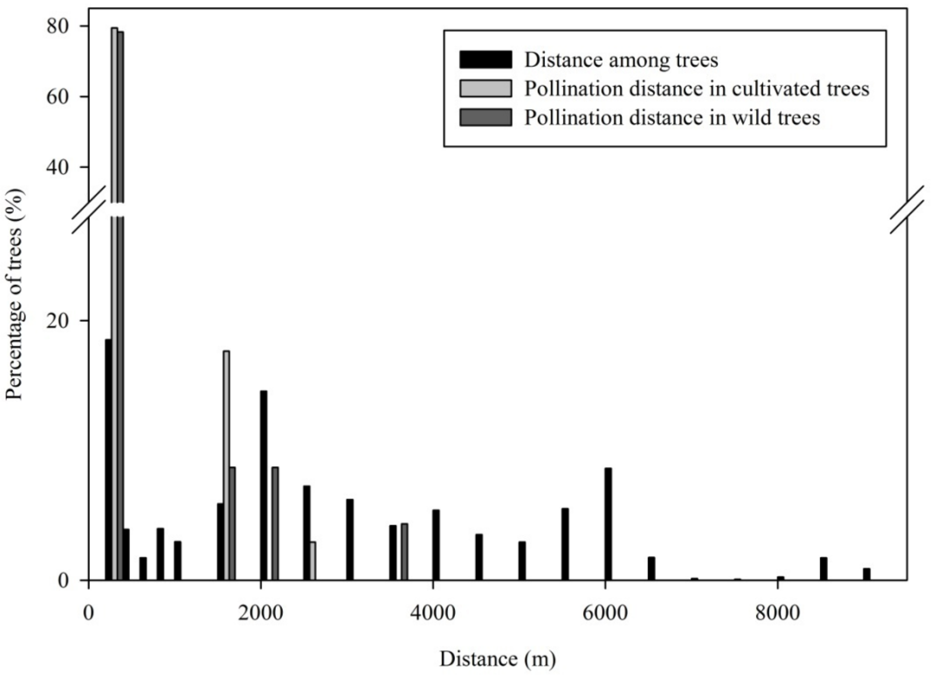

Fig. 2.4 Spatial separation of trees and distributions of pollen dispersal distance in wild and cultivated trees of *Theobroma cacao.* Note that self-pollinated individuals are included.

Considering the origin of the pollen, we found that flowers of wild cacao were mainly pollinated with pollen from wild plants (80%) and flowers of cultivated plants from cultivated plants (84%). Nevertheless, we found bidirectional pollen flow between cultivated and wild cacao, with 16% of cultivated offspring fertilized with pollen from wild trees and 20% of wild offspring resulting from fertilization by pollen from cultivated trees.

DISCUSSION

Our results show that wild and cultivated cacao populations in northwestern Bolivia are genetically distinct, with cultivated trees showing higher genetic diversity. Surprisingly, and in contrast to early assumptions (e.g. Pandey, 1960; Falque *et al.*, 1995), self-pollination occurs at meaningful rates in *T. cacao* especially among the cultivated trees. However, cross-pollination is prevalent and mainly takes place over distances of up to a few hundred meters. Accordingly, pollination mostly occurs within the spatially segregated cultivated and wild populations. However, pollen dispersal over several kilometers was also observed. Within the wild populations consisting of scattered *T. cacao* trees, long distance pollen dispersal can thus play a major role in maintaining genetic connectivity. However pollen dispersal can also lead to gene flow between wild and cultivated populations. This was evidenced by a number of seedlings with mixed parentage as well as admixed adult plants.

Cacao origin—It has been proposed that the "wild" populations of *T. cacao* in Bolivia are the result of naturalized plants from previous cultivation (Tratado de Cooperación Amazónica, 1999). However, our study clearly distinguished between cultivated and "wild" cacao plants (Fig. 2.2) showing that the wild plants are genetically distinct from the currently cultivated ones. Although this is not conclusive evidence, it nevertheless suggests that the "wild" cacao populations of lowland Bolivia may correspond to truly wild forms or at least to ancient cultivars that have become naturalized a long time ago. This lends support to the assumption that Bolivia is part of the natural range of *T. cacao* (Soria, 1969; Whitkus *et al.*, 1998; Arguello *et al.*, 2000; Motamayor *et al.*, 2003; Sereno *et al.*, 2006). Furthermore, we only found few individuals corresponding to the cultivated genotypes in the forest, which shows that currently, seed dispersal of cultivated forms into the forest (either by humans or animals) and successful establishment is infrequent.

Genetic diversity—Genetic diversity was lower among the wild than the cultivated plants. In our study area, cultivated cacao has a higher genetic diversity, probably because the cultivated forms belong to both major cultivars (*Forastero* and *Criollo*) and, presumably, come from a variety of origins. However, genetic variation of the Bolivian populations found in our study was also lower than values reported for wild cacao populations in Brazil (Sereno *et al.*, 2006), both for alleles (A = 26 vs. A = 49) and expected heterozygosity (H_E = 0.391 vs H_E = 0.566). The low diversity of wild cacao populations in Bolivia has previously been deduced, also on morphological grounds, by R. Villegas (Universidad Mayor de San Andrés, unpublished manuscript) and W. July (CATIE, unpublished manuscript), who further suggest that the present-day wild populations may have derived from the propagation of a few plants present in the natural area of distribution. To which degree this reflects human activities or, e.g., natural clines of genetic variation within the natural range of cacao cannot be deduced with the data at hand.

Cross- and self-pollination**—**In contrast to most previous studies that have reported self-incompatibility in *T. cacao* (e.g. Pandey, 1960; Falque, *et al.*, 1995), our results revealed that self-pollination takes place in both wild and cultivated *T. cacao*, even though cross-pollination clearly predominates. Gledinning (1972) and Lanaud *et al.* (1987) already pointed out that the incompatibility system in cacao is not absolute, but quantitative. Lanaud *et al.* (1987) further suggested that geitonogamy might not be rare in the wild, even though they did not study mating systems under natural conditions. Indeed, the low rate of heterozygosity in wild populations might point to high rates of geitonogamy, in agreement with the observations of Sereno *et al.* (2006) in upper Amazonia.

We found self-pollination to be more common in cultivated than in wild cacao trees, despite the close proximity of cultivated plants, which should increase cross-pollination. The higher degree of self-pollination in cultivated cacao may be the result of human selection toward high crop production in cultivated plants. A similar reduction of self-incompatibility in cultivated forms has also been found (e.g., in *Brassica oleracea*; Thompson and Taylor, 1966).

Paternity assignment and pollen dispersal distance —The rate of paternity assignment in our study was low (17%). This may be attributable to three factors. First, a comparative lack of standing genetic variation precludes discrimination among potential fathers. Second, pollen immigration from outside the sampling area is indicated, in that 11% of the alleles were private to the offspring. Third, unsampled fathers within the sampling area may have contributed. Thus, the results of paternity assignments and pollen dispersal distances may be biased to a certain degree. Still, however, both self-pollination and long distance pollen dispersal events are clearly indicated and consistent with the hybrid seed and adult individuals found.

Most pollination events documented in our study took place over distances of less than a few hundred meters, as is typical for many tropical species (e.g., Cloutier *et al.*, 2007; Carneiro *et al.*, 2009). Yet the distance and frequency of long-distance pollen dispersal are strongly relevant in that they connect populations and isolated groups of individuals across the landscape, increase effective population sizes, and, thus, maintain genetic variability and weakening genetic drift (Oddou-Muratorio *et al.*, 2005). Therefore, many authors have stressed the importance of understanding mating systems and gene flow in the context of sustainable management and conservation of tropical forests (e.g., Bawa, 1990; Cloutier *et al.*, 2007).

The mean pollen dispersal distance found by us in *T. cacao* (867 m) was higher than that reported for other insect-pollinated tropical understory tree species. Typically, such species have dispersal distances <600 m, as observed, for example, in *Carapa guianensis* and *Sextonia rubra* (Cloutier *et al.*, 2007), *Micona affinis* (Jha and Dick, 2010), and

Pithecellobium elegans (Chase *et al*., 1996). Furthermore, we documented pollen dispersal distances up to 3 km in single individuals. Such pollen dispersal distances are unusual for an insect-pollinated tropical understory tree species, although distances >2 km have been found in *Symphonia globulifera* (Carneiro *et al*., 2009). In canopy trees, pollen dispersal distances tend to be larger, as exemplified by distances of 5.8-14.2 km in seven species of the genus *Ficus*, which are pollinated by small wasps (Nason *et al*., 1998).

The large pollen dispersal distances in *T. cacao* are surprising, considering the minute sizes of the pollinating ceratopogonid midges (0.5-2 mm). Although dispersal over long distances is poorly understood in insects, individuals of small species of Diptera, Homoptera, and Hymenoptera are known to be wind dispersed (Nason *et al*., 1998). However, such wind dispersal is unlikely in the interior of the forest understory and suggests either that the insects disperse actively or that they emerge over the forest canopy to be transported in the aerial plankton, as also assumed for fig-pollinating wasps (Nason *et al*., 1998).

Crop-wild gene flow—Our results revealed bidirectional pollen flow between wild and cultivated cacao. This was evident both for seeds from the paternity analysis and for adult plants, indicating that hybrids can establish under natural conditions. Pollen flow from crop to wild relatives carries the risk of genetic contamination of wild populations (Ellstrand, et al., 1999; Fénart *et al*., 2007). According to Arias and Reiseberg (1994) and Fénart *et al.* (2007), in general, spatial separation among wild and cultivated forms alone is rarely enough to prevent gene flow. Indeed, considering the large pollen dispersal distances we found in cacao, physical distances of a few hundred meters as in our study cannot totally prevent pollen flow between the cacao forms. Wild populations are considered reservoirs of genetic diversity, but the studied wild populations showed lower genetic diversity than the crops. In this case, gene flow actually could increase the genetic diversity of wild cacao, although long-term consequences are currently unknown and need more study. In Bolivia, most wild populations of *Theobroma cacao* occur in the low-lying Amazonian plain, whereas cacao cultivation mainly takes place on the Andean foothills. Our study region was located right at the boundary of these two zones, indicating that crop-to-wild gene flow is most relevant in areas were both wild and cultivated gene pools are common.

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ACKNOWLEDGMENTS

The authors thank the German Science Foundation (HE 3041/17-1) for funding this project. We also thank the German Academic Exchange Service for the scholarship granted to CC. For assistance in the field, we thank R. Hurtado, C. Campos, and the local assistants J. Navi, C. Ibaguari, E. Amutari, and W. Amutari. Special thanks to B. Müller and S. Träger for the assistance in the laboratory. We also thank the National Herbarium of Bolivia for logistic support, and the Ministerio de Desarrollo Rural y Medio Ambiente – Dirección General de Biodiversidad y Áreas Protegidas in Bolivia for research permits.

APPENDIX 2.1. Allele frequency analysis of wild and cultivated *Theobroma cacao*.

Locus	k	*n*	H_O	H_E	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	F(Null)
Cir6	6	562	0.247	0.331	0.318	0.941	0.811	0.669	0.46	0.7	***	+0.1751
Cir25	14	571	0.578	0.779	0.747	0.6	0.422	0.234	0.081	0.381	ND	+0.1479
Cir11	10	561	0.353	0.592	0.565	0.791	0.609	0.404	0.193	0.503	ND	+0.2535
Cir12	17	571	0.708	0.835	0.814	0.494	0.326	0.151	0.048	0.345	***	+0.0832
Cir7	8	561	0.449	0.646	0.597	0.768	0.599	0.418	0.174	0.471	ND	+0.1728
Cir18	9	571	0.469	0.521	0.496	0.844	0.673	0.48	0.255	0.553	***	+0.0497
Cir1	10	571	0.536	0.619	0.554	0.8	0.649	0.487	0.21	0.494	***	+0.0656
Cir15	14	569	0.343	0.447	0.423	0.889	0.734	0.561	0.33	0.609	***	+0.1477
Cir21	11	568	0.41	0.623	0.603	0.756	0.565	0.348	0.162	0.479	***	+0.2378

Note. 572 samples of adults and offspring. *K* = number of alleles; *n* = number of individuals; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content; NE-1P = combined non exclusion probability (first parent); NE-2P = combined non exclusion probability (second parent); NE-PP = combined non exclusion probability (parent pair); NE-I = combined non exclusion probability (identity); HW = Hardy Weinberg; F(Null) = frequency of null allele.

APPENDIX 2.2. Paternity assignment in *Theobroma cacao* (mean observed error rate across loci = 0.1166; dispersal distance range: 10 – 3007 m).

Level	Confidence (%)	Critical Delta	Assignments		Assignment rate	
			Observed	Expected	Observed	Expected
Strict	95	5.59	44	73	13%	21%
Relaxed	80	2.13	57	107	17%	31%

APPENDIX 2.3. Summary of private alleles in parental trees and offspring of *Theobroma cacao*.

Type	Locus	Allele	Frequency
Parental tree	mTcCir25	132	0.007
Parental tree	mTcCir25	142	0.024
Parental tree	mTcCir25	160	0.007
Parental tree	mTcCir25	176	0.002
Parental tree	mTcCir11	300	0.002
Parental tree	mTcCir12	184	0.002
Parental tree	mTcCir12	192	0.002
Parental tree	mTcCir12	210	0.002
Parental tree	mTcCir1	136	0.002
Parental tree	mTcCir1	140	0.002
Parental tree	mTcCir1	148	0.002
Parental tree	mTcCir1	152	0.002
Parental tree	mTcCir15	244	0.009
Parental tree	mTcCir15	246	0.013
Parental tree	mTcCir15	248	0.002
Parental tree	mTcCir15	250	0.004
Parental tree	mTcCir21	184	0.004
Offspring	mTcCir11	296	0.003
Offspring	mTcCir12	194	0.001
Offspring	mTcCir12	198	0.001
Offspring	mTcCir12	200	0.001
Offspring	mTcCir7	148	0.005
Offspring	mTcCir7	152	0.002
Offspring	mTcCir7	164	0.002
Offspring	mTcCir7	166	0.003
Offspring	mTcCir18	330	0.001
Offspring	mTcCir18	350	0.006
Offspring	mTcCir1	144	0.003

APPENDIX 2.4. Genotype of *Theobroma cacao* adult trees

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E001	Wild	232	232	134	140	292	292	212	220	160	160	344	348	142	146	276	276	236	236	160	170
E002	Wild	232	234	138	150	306	306	202	212	156	156	338	344	130	130	272	276	240	258	158	158
E003	Wild	232	232	134	140	292	292	212	220	160	160	344	344	142	146	276	276	236	236	170	170
E004	Wild	232	232	134	136	292	292	212	220	160	160	344	344	146	146	276	276	236	236	170	170
E005	Wild	232	232	134	140	292	292	220	246	160	162	344	344	146	146	276	276	236	256	170	170
E006	Wild	232	232	140	140	292	292	212	220	160	160	344	344	142	146	276	276	236	236	170	170
E007	Wild	232	232	134	140	292	292	212	236	160	160	344	344	142	146	274	276	236	236	170	170
E008	Wild	232	232	140	140	292	292	220	246	162	162	344	344	142	142	276	276	236	236	170	170
E009	Wild	232	232	134	134	292	292	212	212	160	160	344	344	142	150	276	276	236	236	170	170
E010	Wild	232	232	140	140	292	292	204	204	162	162	344	344	146	146	274	276	236	236	170	170
E011	Wild	232	232	134	148	292	292	220	246	162	162	344	344	142	146	276	276	236	252	170	170
E012	Wild	232	232	140	140	292	292	220	220	160	160	344	348	146	146	276	278	236	236	170	170
E013	Wild	232	232	134	134	292	292	212	246	160	162	344	344	142	142	276	276	236	236	170	170
E014	Wild	232	232	134	134	292	292	212	236	162	162	344	344	142	150	276	276	236	236	170	170
E015	Wild	232	232	140	140	290	292	220	220	160	162	344	344	142	146	276	276	236	250	170	172
E016	Wild	232	232	140	140	292	310	220	246	160	160	344	344	142	146	276	276	236	236	160	170
E017	Cult	232	234	150	150	304	306	190	202	156	160	336	346	142	142	274	274	238	256	148	168
E018	Cult/h	228	232	138	154	292	292	214	220	156	162	334	344	130	142	274	276	236	248	158	170
E019	Wild	232	232	134	140	292	292	212	246	160	162	344	344	142	142	276	276	236	236	148	170

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E020	Wild	232	232	134	134	292	310	212	212	158	160	348	348	146	146	276	276	236	236	170	170
E021	Wild	232	232	134	134	292	292	220	236	160	162	348	348	142	146	276	276	236	236	170	170
E022	Wild	232	232	136	140	292	292	220	236	158	160	344	348	142	146	276	276	236	252	170	170
E023	Wild	232	232	134	136	290	292	220	236	160	160	344	348	142	146	276	276	236	236	170	170
E024	Wild	232	232	136	140	292	292	220	236	158	160	344	348	142	146	276	276	236	252	170	170
E025	Wild	232	232	134	136	292	292	220	246	160	162	344	348	142	146	276	276	236	236	170	170
E026	Wild	232	232	134	140	0	0	236	236	160	160	344	348	142	142	276	276	236	236	170	170
E027	Wild	232	232	134	140	292	310	220	246	158	160	348	348	142	150	276	276	236	236	170	170
E028	Wild	232	232	134	136	290	292	212	212	160	160	344	344	142	146	276	276	252	260	160	170
E029	Wild	232	232	134	136	290	292	236	246	158	160	344	344	146	146	276	276	252	252	170	170
E030	Wild	232	232	136	140	292	292	220	220	158	160	344	348	146	146	276	276	236	252	170	170
E031	Wild	232	232	134	140	292	292	212	220	158	160	344	344	146	146	276	276	236	252	160	170
E032	Wild	232	232	134	140	290	310	246	246	160	162	344	344	142	146	276	276	236	260	170	170
E033	Wild	232	232	134	140	290	292	220	220	160	160	344	348	146	150	276	276	252	260	170	170
E034	Wild	232	232	136	140	292	292	204	236	160	162	344	348	146	146	276	276	236	236	170	172
E035	Wild	232	232	136	140	292	292	236	246	160	160	348	348	146	146	276	276	236	236	170	170
E036	Wild	232	232	134	136	292	292	212	220	160	162	344	344	142	142	276	276	236	236	170	170
E037	Wild	232	232	134	134	292	292	220	236	160	162	344	344	142	146	276	276	236	236	170	170
E038	Wild	232	232	134	140	292	292	236	246	160	162	344	344	142	146	276	276	236	236	170	172
E039	Wild	232	232	134	140	292	292	212	220	160	162	344	344	142	146	276	276	236	236	170	170

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E040	Wild	232	232	140	140	292	292	212	220	160	162	344	344	142	146	276	276	236	236	170	170
E041	Wild	232	232	134	140	292	292	220	220	160	162	344	344	142	142	276	276	236	236	170	170
E042	Wild	232	232	134	134	292	292	220	236	160	162	344	344	142	142	276	276	236	236	170	170
E043	Wild	232	232	134	134	292	310	220	220	160	160	344	348	142	146	274	276	236	236	170	172
E044	Wild	232	232	134	140	292	292	212	246	158	160	344	344	146	146	276	276	236	236	170	170
E045	Wild	232	232	140	140	292	292	212	220	160	162	344	344	146	146	276	276	236	236	170	170
E046	Wild	232	232	136	140	292	292	236	246	160	160	344	344	142	146	274	276	236	236	170	170
E047	Wild	232	232	134	134	292	292	236	236	160	160	344	344	146	150	276	276	236	236	160	170
E048	Wild	232	232	134	134	290	290	220	246	162	162	344	344	142	150	276	276	236	236	170	170
E049	Wild	232	232	134	134	292	292	246	246	160	160	344	344	142	142	276	276	236	236	170	170
E050	Wild	232	232	134	140	292	292	220	246	158	160	344	344	142	146	276	276	236	236	170	172
E051	Wild	232	232	136	140	292	292	220	236	160	160	344	344	142	146	276	276	236	236	170	170
E052	Wild	232	232	134	140	292	310	212	220	160	160	344	348	142	146	276	276	236	236	170	170
E053	Wild	232	232	134	140	292	310	220	236	158	158	344	348	142	146	276	276	236	236	170	170
E054	Wild	232	232	134	140	292	292	220	246	158	160	344	344	142	142	276	276	236	236	170	170
E055	Wild	232	232	140	140	292	292	212	220	158	162	344	344	142	142	276	276	236	236	170	170
E056	Wild	232	232	134	134	290	292	210	220	160	162	348	348	142	142	276	276	236	236	170	172
E058	Wild	232	232	140	140	290	310	220	220	158	160	344	344	142	146	276	276	236	236	170	172
E059	Wild	232	232	134	140	290	290	236	246	160	160	344	348	142	142	276	276	236	236	170	172
E060	Wild	232	232	140	140	292	292	220	236	158	162	344	344	142	142	276	276	236	236	170	172

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E061	Wild	232	232	140	140	292	292	220	220	160	160	344	344	142	142	276	276	236	236	170	170
E062	Wild	232	232	140	140	292	292	220	220	160	160	344	344	142	142	276	276	236	236	170	170
E063	Wild	232	232	134	134	292	292	236	236	162	162	344	344	142	142	276	276	236	236	170	172
E064	Wild	232	232	134	140	290	292	220	246	160	162	344	348	142	142	276	276	236	236	170	172
E065	Wild	232	232	134	136	292	292	220	220	158	160	344	348	142	142	276	276	236	236	170	170
E066	Wild	232	232	134	140	290	292	220	220	162	162	344	348	142	142	276	276	236	236	170	170
E067	Wild	232	232	134	140	292	292	236	246	160	160	344	344	146	146	276	276	236	236	170	172
E068	Wild	232	232	134	136	292	292	220	236	160	160	344	344	142	142	276	276	236	236	170	172
E069	Wild	232	232	148	148	292	292	220	246	160	162	344	344	142	142	276	276	236	256	170	170
E070	Wild	232	232	134	134	292	292	246	246	158	162	344	344	142	142	276	276	236	236	170	172
E071	Wild	232	232	134	148	292	292	212	212	160	162	344	348	146	146	276	276	236	236	170	170
E072	Wild	232	232	134	140	292	292	220	246	160	162	344	344	142	148	276	276	236	236	170	170
E073	Wild	232	232	140	140	292	292	220	236	160	162	344	344	142	146	276	276	236	250	170	172
E074	Wild.cult	232	232	134	154	292	292	220	220	160	160	344	344	142	142	276	276	236	236	170	170
E075	Wild.cult	232	232	134	134	292	292	220	220	160	162	348	348	146	146	276	276	236	236	170	170
E076	Wild.cult	232	232	134	134	292	292	212	220	162	162	344	344	146	146	276	276	236	236	170	170
E077	Wild.cult	232	232	140	150	292	292	246	246	162	162	344	344	146	146	274	276	236	252	170	170
E078	Wild.cult	232	232	134	150	292	292	220	220	160	162	344	344	142	146	276	276	236	236	160	170
E079	Wild.cult	232	232	140	140	292	292	220	220	162	162	348	348	142	142	276	276	236	256	170	170
E080	Wild.cult	232	232	140	140	290	292	220	220	160	160	344	348	142	142	276	276	236	236	170	170

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E081	Wild.cult	232	232	134	140	292	292	212	220	158	162	344	344	142	146	276	276	236	236	170	170
E082	Wild.cult	232	232	136	140	290	290	220	220	160	162	344	344	142	146	276	276	236	236	170	170
E083	Wild.cult	232	232	140	148	292	292	212	246	160	162	344	348	142	142	276	276	236	254	170	170
E084	Wild.cult	232	232	134	140	292	292	0	0	160	160	344	348	142	142	276	276	236	236	160	160
E087	Wild.cult	232	232	134	136	292	292	212	236	162	162	344	344	142	146	276	276	236	236	170	170
E088	Wild.cult	232	232	136	136	292	292	220	246	162	162	344	344	130	142	276	276	236	236	160	170
E086	Cult	232	242	138	150	306	306	206	212	160	160	338	344	130	142	272	274	236	238	148	148
E089	Cult	232	242	136	150	292	292	190	206	156	160	334	356	142	142	274	274	236	260	148	176
E090	Cult	228	232	142	150	310	318	202	212	156	162	336	344	130	130	274	274	238	246	154	158
E144	Cult	232	242	138	150	318	318	202	212	160	160	338	344	130	142	276	276	236	242	146	148
E095	Cult	232	234	150	156	318	318	206	212	160	160	336	344	130	142	272	274	236	242	148	148
E096	Cult	228	232	136	138	318	318	190	214	160	162	334	336	130	130	274	274	238	246	148	158
E097	Cult	232	242	138	150	306	306	202	212	160	160	338	344	130	142	272	274	236	238	146	158
E098	Cult	228	232	142	150	318	318	190	202	156	162	334	336	142	142	274	274	236	246	154	158
E099	Cult	232	242	138	138	310	310	206	212	160	160	344	356	140	142	274	274	238	254	158	184
E100	Cult	228	232	138	142	318	318	190	202	156	162	334	336	130	142	274	274	238	254	158	158
E102	Cult	228	228	0	0	0	0	212	212	156	156	344	346	130	142	274	274	236	238	146	158
E103	Cult	232	240	138	160	292	292	212	214	160	160	336	344	142	142	276	276	238	238	148	154
E104	Cult	228	242	138	156	318	318	206	212	160	160	338	344	130	142	272	274	236	240	148	158
E105	Cult	232	242	138	150	318	318	202	212	160	160	338	344	130	142	274	276	236	238	146	158

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E106	Cult	228	242	138	156	306	306	206	212	160	160	336	344	130	142	276	276	236	238	146	148
E107	Cult	232	234	138	150	318	318	206	212	160	160	338	344	130	142	272	274	236	238	148	148
E108	Cult	228	232	142	150	318	318	212	214	160	160	336	344	130	130	274	274	236	244	148	158
E109	Cult	228	232	138	142	310	310	212	214	160	160	336	344	142	142	274	274	238	254	148	158
E110	Cult	228	232	138	142	310	310	212	214	160	162	336	344	142	142	274	274	236	246	148	154
E111	Cult	232	242	138	156	318	318	206	212	160	160	338	344	130	142	272	274	236	238	146	158
E112	Cult	232	234	138	138	318	318	206	212	160	160	338	344	130	142	272	274	236	238	146	148
E113	Cult	232	242	138	156	306	306	206	212	160	160	338	344	130	142	272	274	236	238	148	148
E114	Cult	228	234	138	150	306	306	202	212	160	160	336	344	130	142	274	274	236	238	146	158
E115	Cult	232	242	150	160	292	292	206	212	160	160	334	356	142	142	274	274	236	238	148	176
E116	Cult	232	242	138	150	310	318	206	212	160	160	344	356	130	142	274	274	236	260	148	184
E117	Cult	232	242	138	150	318	318	206	212	160	160	336	344	130	142	274	274	236	240	146	148
E118	Cult	228	232	142	150	318	318	190	214	160	160	334	336	130	142	274	274	238	246	158	158
E119	Cult	228	232	142	150	310	310	202	212	160	162	336	344	130	130	274	274	236	244	158	158
E120	Cult	228	232	142	150	318	318	212	214	156	162	336	344	130	142	274	274	236	244	154	158
E121	Cult	232	242	138	138	306	306	202	212	160	160	334	344	130	142	274	274	236	238	146	158
E122	Cult	232	234	138	138	306	306	202	212	160	160	334	344	130	142	274	274	236	238	148	158
E123	Cult	232	242	138	138	318	318	202	212	160	160	336	344	130	142	274	274	236	238	146	148
E124	Cult	228	242	138	138	318	318	206	212	160	160	338	344	130	142	272	274	236	238	146	158
E125	Wild.cult	232	232	148	148	292	292	204	220	158	162	344	344	142	146	274	276	234	236	170	170

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E126	Wild.cult	232	232	134	134	292	292	220	220	160	160	344	348	142	146	276	276	236	236	170	170
E127	Wild.cult	232	232	134	140	292	292	220	220	158	158	344	344	146	146	276	276	236	236	170	170
E128	Wild.cult	232	232	134	136	292	292	212	236	160	160	344	344	142	146	276	276	236	252	170	170
E129	Wild.cult	232	232	134	154	292	292	204	236	158	160	344	348	142	146	276	276	236	236	170	170
E130	Wild.cult	232	232	134	140	290	292	220	220	162	162	344	344	142	142	274	276	236	236	170	170
E131	Wild.cult	232	232	140	140	290	292	204	220	160	160	344	344	146	146	276	276	236	236	170	170
E132	Wild.cult	232	232	134	136	290	292	220	246	160	162	344	344	130	146	276	276	236	236	170	172
E133	Wild.cult	232	232	134	134	292	292	220	236	160	160	344	344	146	146	276	276	236	236	170	170
E134	Wild.cult	232	232	140	140	290	292	220	246	160	162	344	344	146	146	276	276	236	236	170	170
E135	Wild.cult	232	232	134	140	290	292	212	220	160	160	344	344	142	146	276	276	236	236	170	170
E136	Wild.cult	232	232	140	140	292	292	218	246	160	160	344	344	146	150	276	276	236	236	170	170
E137	Wild.cult	232	232	134	136	290	292	204	212	160	160	344	344	142	146	276	276	236	236	170	170
E138	Wild.cult	232	232	136	136	292	292	220	246	160	160	344	344	132	146	276	276	236	236	170	170
E139	Wild.cult	232	232	140	140	292	292	220	246	160	162	344	344	142	146	276	276	236	236	170	170
E140	Wild.cult	232	232	134	140	292	292	220	246	160	162	344	344	146	146	276	276	236	236	170	170
E141	Wild.cult	232	232	134	134	290	292	204	246	160	162	344	344	142	146	276	276	236	236	170	170
E142	Wild.cult	232	232	148	148	290	292	246	246	158	162	344	344	130	142	276	276	236	236	160	170
E143	Wild.cult	232	232	140	140	292	292	236	246	160	162	344	344	142	142	276	276	236	236	160	172
E091	Wild.cult	232	232	134	140	292	292	204	236	162	162	344	348	142	146	276	276	236	236	170	172
E092	Wild.cult	232	232	134	140	292	292	236	236	160	162	344	344	142	146	276	276	236	236	170	172

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
E093	Wild.cult	232	232	140	148	292	292	246	246	160	162	344	344	142	146	276	276	236	236	170	170
E094	Wild.cult	232	232	140	140	290	292	220	220	160	160	344	344	142	142	276	276	236	236	170	170
A001	Wild.cult	232	232	134	140	292	292	246	246	160	162	344	344	142	146	276	276	236	236	160	170
A002	Wild.cult	232	232	140	148	290	292	246	246	158	160	344	344	142	146	276	276	236	236	160	170
A003	Wild.cult	232	232	148	148	290	292	220	246	158	160	344	344	142	142	274	276	236	236	170	170
A004	Wild.cult	232	232	140	148	292	292	220	246	158	160	344	344	130	142	276	276	236	236	160	170
A005	Wild.cult	232	232	134	134	292	292	246	246	158	158	344	344	142	142	276	276	236	236	172	172
A006	Wild.cult	232	232	134	140	292	302	204	246	158	158	344	348	142	142	276	276	236	236	160	172
A007	Wild.cult	232	232	126	130	292	292	204	246	158	158	344	348	142	142	276	276	236	236	172	172
A008	Wild.cult	232	232	132	140	292	302	212	246	158	158	344	348	142	142	276	276	236	236	170	172
A009	Wild.cult	232	232	134	140	292	292	246	246	160	162	344	344	142	142	276	276	236	236	170	170
A010	Wild.cult	232	232	140	148	290	292	220	246	160	162	344	344	130	146	276	276	236	236	160	170
A011	Cult	228	232	136	138	318	318	190	214	160	162	336	336	130	130	274	274	236	246	158	158
A012	Cult	232	234	138	150	318	318	206	212	160	160	338	344	130	142	272	274	236	242	146	148
A013	Cult	232	242	138	156	318	318	206	212	160	160	336	344	130	142	274	274	236	238	148	148
A014	Cult	228	228	140	150	318	318	190	214	160	160	336	344	142	142	274	274	238	254	148	158
A015	Cult	228	234	138	138	306	306	202	212	160	160	336	344	130	142	274	274	236	238	146	148
A016	Cult	228	242	138	156	318	318	206	212	160	160	338	344	130	142	272	274	236	238	146	148
A017	Cult	228	242	138	156	306	306	206	212	160	160	338	344	130	142	272	274	236	242	148	148
A018	Cult	232	242	138	138	318	318	206	212	160	160	338	344	130	142	272	274	236	238	146	158

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
A019	Wild.cult	232	232	134	140	290	292	212	236	160	160	344	348	142	146	276	276	236	236	170	170
A020	Cult	232	242	150	160	300	304	184	192	160	160	334	356	130	142	274	274	238	254	158	158
A021	Cult	232	232	142	150	304	304	202	214	156	160	344	346	130	130	274	274	236	238	146	158
A022	Cult	232	250	150	156	318	318	206	212	156	160	344	346	130	130	274	274	238	256	146	158
A023	Cult	232	234	150	156	310	310	214	214	156	160	344	344	142	142	274	274	236	244	146	148
A024	Cult/h	232	232	134	138	292	292	212	212	160	162	344	344	130	146	274	276	236	238	148	170
A025	Cult	232	232	150	150	318	318	204	212	156	160	336	346	130	142	274	274	238	238	158	160
A030	Cult	228	242	138	138	318	318	190	212	156	160	344	346	130	130	274	274	238	258	168	168
A029	Cult	228	242	136	142	302	302	190	216	160	160	344	346	130	130	274	276	236	254	152	168
A028	Cult	228	240	138	150	292	292	212	214	156	156	344	344	130	142	274	274	238	238	146	176
A026	Cult/h	232	232	134	138	292	318	206	246	160	162	338	344	142	146	272	276	236	242	146	170
A027	Cult	228	232	150	150	304	318	212	214	160	160	344	344	130	130	274	274	238	258	158	168
A031	Cult	232	242	138	140	292	318	214	220	156	162	336	344	142	146	274	276	236	238	160	170
A032	Cult	232	250	140	150	304	304	202	212	156	162	344	346	130	142	276	276	236	242	148	158
A033	Cult	228	232	136	150	310	318	212	216	160	160	344	344	130	130	276	276	238	238	148	152
A034	Cult	228	232	134	150	292	292	190	214	156	162	344	346	130	146	274	276	236	238	168	170
A035	Cult	234	234	150	150	302	302	206	206	156	160	338	346	130	136	274	290	236	256	168	172
A036	Wild.cult	232	232	140	140	292	292	220	220	162	162	344	344	142	146	276	276	234	236	170	170
A037	Cult	232	232	138	156	304	304	212	214	156	156	336	344	130	152	274	274	236	256	160	168
A038	Cult/h	232	232	134	156	292	292	206	212	160	162	338	344	142	146	272	276	236	238	158	170

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
A039	Cult	232	240	138	150	292	306	212	214	160	160	334	344	130	142	274	284	238	256	146	176
A040	Cult	232	232	138	140	292	292	212	214	156	162	344	346	142	146	274	276	236	238	168	170
A041	Cult	232	242	138	150	304	318	190	202	156	160	338	346	130	142	272	274	238	238	158	168
A042	Cult	232	232	138	150	318	318	190	214	156	160	344	346	130	142	274	274	236	238	146	158
A043	Cult	234	250	138	150	318	318	202	214	156	160	338	346	142	142	272	274	238	242	148	158
A044	Cult	234	250	150	150	306	318	190	202	156	160	334	338	130	142	274	284	238	256	158	158
A045	Cult	232	250	138	150	318	318	212	214	156	160	344	346	130	142	274	274	236	256	146	158
A046	Cult	232	242	138	150	318	318	190	206	156	160	334	336	130	142	274	284	238	238	148	168
A048	Cult	242	250	138	150	318	318	206	246	156	160	334	338	130	142	274	284	238	256	148	158
A047	Cult	242	242	138	150	318	318	190	202	156	160	338	346	130	142	272	274	242	256	158	168
A049	Cult	228	232	138	150	304	304	212	214	156	160	344	346	130	130	274	274	236	238	148	158
A050	Cult	234	234	150	150	306	318	202	214	156	160	334	338	142	142	272	284	238	242	148	158
A070	Wild	232	232	134	140	292	292	212	236	160	162	344	344	142	146	274	276	236	260	170	170
A052	Wild	232	232	136	140	292	292	220	220	160	160	344	344	146	150	276	276	236	236	170	170
A069	Wild	232	232	134	140	292	310	212	236	160	160	344	348	142	142	276	276	236	236	170	170
A061a	Wild	232	232	134	136	292	292	220	236	160	160	344	348	142	146	276	276	236	236	170	170
A058	Wild	232	232	140	150	290	292	212	246	162	162	344	348	142	150	276	276	236	236	170	170
A067	Wild	232	232	134	134	292	292	212	220	160	160	344	344	146	146	276	276	236	236	170	170
A057	Wild	232	232	136	140	290	292	220	236	160	160	344	344	142	146	276	276	252	252	170	170
A062	Wild	232	232	134	134	292	294	220	236	160	162	344	348	142	142	276	276	236	236	170	170

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
A055	Wild	232	232	134	140	292	310	220	246	160	160	344	348	142	146	276	276	236	236	170	170
A068	Wild	232	232	134	140	290	310	220	220	162	162	344	344	146	146	276	276	236	236	170	170
A061	Wild	232	232	136	140	292	292	212	220	160	160	348	348	142	146	276	276	236	236	170	170
A066	Wild	232	232	134	140	292	292	220	220	160	160	344	344	142	146	276	276	254	260	170	170
A065	Wild	232	232	134	140	292	310	220	246	160	160	344	344	142	142	276	276	236	236	170	172
A060	Wild	232	232	134	140	290	292	212	220	160	162	344	344	142	146	276	276	236	236	170	170
A064	Wild	232	232	134	140	292	294	220	220	158	162	344	344	142	146	274	276	236	236	170	170
A059	Wild	232	232	134	140	292	292	212	220	160	162	344	348	142	146	276	276	236	236	170	170
A063	Wild	232	232	134	140	292	292	212	220	158	158	344	344	142	146	276	276	236	236	170	170
A054	Wild	232	232	132	138	290	292	212	220	160	160	344	344	142	150	276	276	236	236	170	170
A056	Wild	232	232	134	140	292	292	220	236	160	160	344	344	142	146	276	276	236	236	170	170
A053	Wild	232	232	134	140	292	292	212	220	160	160	344	344	142	146	276	276	236	252	170	170
A051	Wild	232	232	134	140	290	292	204	220	162	162	344	344	142	146	276	276	236	236	172	172
A074	Wild	232	232	134	140	292	292	212	236	160	162	348	348	142	142	276	276	236	236	170	170
A075	Wild	232	232	134	140	292	310	204	212	158	162	344	348	142	146	276	276	234	236	170	170
A072	Wild	232	232	134	134	292	292	236	246	160	160	344	344	142	146	276	276	236	236	170	170
A079	Wild	232	232	134	176	290	290	220	236	160	160	344	344	142	142	276	276	236	236	170	172
A076	Wild	232	232	134	134	292	292	220	236	160	160	344	344	142	146	276	276	236	236	170	170
A084	Wild	232	232	134	140	292	292	220	220	160	160	344	344	142	146	276	276	236	236	170	170
A086	Wild	232	232	134	140	292	292	220	236	158	160	344	348	142	146	276	276	236	236	170	170

ID	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
A077	Wild	232	232	134	134	292	292	236	246	160	162	344	348	142	146	276	276	236	236	170	172
A082	Wild	232	232	134	134	292	292	220	220	158	160	344	344	142	142	276	276	236	236	170	170
A087	Wild	232	232	134	140	290	292	236	246	160	162	344	348	142	146	276	276	236	236	170	170
A078	Wild	232	232	134	140	292	310	220	236	160	162	344	344	142	146	276	276	236	236	172	172
A090	Wild	232	232	134	136	292	292	212	236	160	160	344	344	142	146	276	276	236	236	170	172
A071	Wild	232	232	134	140	292	310	212	246	160	160	344	344	146	146	276	276	236	236	170	170
A088	Wild	232	232	140	140	292	292	212	212	158	160	344	344	142	146	276	276	236	236	170	170
A085	Wild	232	232	132	140	292	292	212	236	160	160	344	348	142	142	276	276	236	236	170	170
A083	Wild	232	232	140	140	292	310	220	236	160	160	348	348	142	146	276	276	236	236	170	170
A073	Wild	232	232	134	140	292	292	212	236	160	160	344	348	142	142	276	276	236	236	170	170

Note. Cult = cultivated; Cult/h = putative hybrids; Wild.cult = cultivated wild cacao

APPENDIX 2.5. Genotype of *Theobroma cacao* offspring

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
194	A001	Wild.cult	232	232	140	140	292	292	246	246	160	160	344	344	144	144	276	276	0	0	0	0
210	A001	Wild.cult	232	232	134	140	292	292	246	246	160	162	344	344	142	146	276	276	236	236	160	160
211	A001	Wild.cult	232	232	134	140	292	292	246	246	162	162	344	344	142	146	276	276	236	236	170	170
212	A001	Wild.cult	232	232	140	140	292	292	246	246	160	162	344	344	146	146	276	276	236	236	160	170
213	A001	Wild.cult	232	232	134	140	292	292	246	246	160	162	344	344	142	146	276	276	236	236	160	170
529	A001	Wild.cult	232	232	134	140	292	292	246	246	162	162	344	344	142	142	276	276	236	236	160	160
530	A001	Wild.cult	232	232	134	134	292	292	246	246	0	0	344	344	142	142	276	276	236	236	170	170
531	A001	Wild.cult	232	232	140	140	292	292	246	246	0	0	344	344	142	146	276	276	236	236	160	160
532	A001	Wild.cult	0	0	140	140	292	292	246	246	0	0	344	344	146	146	276	276	236	236	160	170
533	A001	Wild.cult	232	232	134	134	292	292	246	246	160	160	344	344	142	146	276	276	236	236	160	170
534	A001	Wild.cult	232	232	134	140	292	292	246	246	160	162	344	344	142	146	274	274	236	236	160	170
535	A001	Wild.cult	232	232	140	140	292	292	246	246	162	162	344	344	142	146	276	276	236	236	160	170
536	A001	Wild.cult	232	232	134	134	292	292	246	246	162	162	344	344	142	146	276	276	236	236	160	160
537	A001	Wild.cult	232	232	134	134	292	292	246	246	162	162	344	344	142	146	276	276	236	236	170	170
538	A001	Wild.cult	232	232	134	140	292	302	212	246	158	160	344	344	142	146	276	276	236	236	170	170
214	A002	Wild.cult	232	232	140	140	292	292	246	246	158	160	344	344	142	146	276	276	236	236	160	170
215	A002	Wild.cult	232	232	148	148	290	292	246	246	158	158	344	344	142	146	276	276	236	236	160	160
216	A002	Wild.cult	232	232	148	148	290	292	246	246	158	158	344	344	146	146	276	276	236	236	170	170
217	A002	Wild.cult	0	0	140	148	290	292	246	246	158	158	344	344	142	146	276	276	236	236	160	160

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
539	A002	Wild.cult	232	232	136	148	290	292	220	246	158	160	344	344	142	146	276	276	236	236	170	170
540	A002	Wild.cult	232	232	136	148	290	292	246	246	160	160	344	344	146	146	276	276	236	236	160	170
541	A002	Wild.cult	232	232	136	140	292	292	220	246	158	160	344	344	146	146	276	276	236	236	170	170
542	A002	Wild.cult	232	232	136	148	292	292	246	246	160	160	344	344	146	146	276	276	236	236	160	170
543	A002	Wild.cult	232	232	136	148	292	292	246	246	158	160	344	344	142	146	276	276	236	236	170	170
544	A002	Wild.cult	232	232	136	140	290	292	220	246	158	160	344	344	146	146	276	276	236	236	170	170
545	A002	Wild.cult	232	232	136	148	292	292	220	246	160	160	344	344	146	146	276	276	236	236	160	170
546	A002	Wild.cult	232	232	134	148	292	292	246	246	160	162	344	344	146	146	276	276	236	236	170	170
547	A002	Wild.cult	0	0	140	150	292	306	206	246	158	158	338	344	130	146	272	276	236	236	158	170
548	A002	Wild.cult	232	234	140	150	292	292	206	246	160	160	344	344	142	146	274	274	236	236	158	170
549	A003	Wild.cult	0	0	140	140	290	290	246	246	162	162	344	344	142	142	276	276	236	236	170	170
550	A003	Wild.cult	232	232	140	148	290	292	220	246	0	0	344	344	142	142	274	276	236	236	170	170
551	A003	Wild.cult	232	232	140	140	290	292	246	246	160	162	344	344	142	142	274	274	236	236	170	170
552	A003	Wild.cult	232	232	140	148	290	290	246	246	0	0	344	344	142	142	274	274	236	236	170	170
553	A003	Wild.cult	232	232	140	148	292	292	246	246	160	160	344	344	142	142	274	276	236	236	170	170
554	A003	Wild.cult	232	232	148	148	290	292	246	246	160	160	344	344	142	142	274	274	236	236	170	170
555	A003	Wild.cult	232	232	140	148	290	292	220	246	160	162	344	344	142	142	274	276	236	236	170	170
556	A003	Wild.cult	232	232	148	148	290	292	220	220	160	160	344	344	142	142	274	276	236	236	170	170
557	A003	Wild.cult	232	232	148	148	290	292	220	220	148	148	344	344	142	142	274	274	236	236	170	170
558	A003	Wild.cult	0	0	140	148	290	292	220	246	0	0	344	344	142	142	274	274	236	236	170	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
218	A004	Wild.cult	232	232	138	148	292	292	212	220	158	162	344	344	142	142	276	276	236	252	154	160
219	A004	Wild.cult	232	232	138	140	292	292	212	220	160	160	344	344	130	142	276	276	236	252	148	160
220	A004	Wild.cult	0	0	136	140	292	318	202	220	160	160	336	344	142	142	274	276	236	252	148	170
221	A004	Wild.cult	232	232	138	140	292	318	212	246	160	160	344	344	130	142	276	276	236	236	154	160
222	A004	Wild.cult	232	232	138	148	292	292	202	220	160	162	336	344	130	142	274	276	236	238	154	160
559	A004	Wild.cult	232	232	140	148	292	292	220	236	158	162	344	348	142	146	276	276	236	236	170	170
560	A004	Wild.cult	232	232	148	148	292	292	246	246	160	162	344	348	142	146	276	276	236	236	170	170
561	A004	Wild.cult	232	232	148	148	292	292	246	246	158	158	344	344	142	146	276	276	236	236	160	170
562	A004	Wild.cult	232	232	140	154	292	292	220	236	160	162	344	348	142	146	276	276	236	236	160	172
563	A004	Wild.cult	232	232	148	148	292	292	236	246	158	162	344	348	142	146	276	276	236	236	170	170
564	A004	Wild.cult	232	232	140	154	292	292	220	236	160	162	344	348	142	146	276	276	236	236	170	170
565	A004	Wild.cult	232	232	148	148	292	292	236	246	158	158	344	348	130	146	276	276	236	236	170	170
566	A004	Wild.cult	232	232	140	148	292	292	246	246	160	162	344	344	130	146	276	276	236	236	160	170
567	A004	Wild.cult	232	232	140	148	292	292	220	246	158	160	344	344	130	146	276	276	236	236	170	172
568	A004	Wild.cult	232	232	148	154	292	292	236	246	160	162	344	348	130	146	276	276	236	236	160	170
569	A006	Wild.cult	232	232	134	134	292	302	204	246	158	158	344	348	142	142	276	276	236	236	160	160
570	A006	Wild.cult	232	232	140	140	302	302	204	204	158	158	344	344	142	142	276	276	236	236	160	160
571	A006	Wild.cult	232	232	134	140	292	292	204	246	158	158	344	348	142	142	276	276	236	236	172	172
572	A006	Wild.cult	232	232	134	140	302	302	204	246	158	158	348	348	142	142	276	276	236	236	160	160
573	A006	Wild.cult	232	232	140	140	292	302	204	246	158	158	344	348	142	142	276	276	236	236	160	172

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
574	A006	Wild.cult	232	232	134	134	292	292	204	246	158	158	344	348	142	142	276	276	236	236	160	172
575	A006	Wild.cult	232	232	134	140	292	302	246	246	158	158	348	348	142	142	276	276	236	236	160	172
576	A006	Wild.cult	232	232	134	134	302	302	246	246	158	158	348	348	142	142	276	276	236	236	172	172
577	A006	Wild.cult	232	232	134	140	292	302	204	204	158	158	344	344	142	142	276	276	236	236	160	160
578	A006	Wild.cult	232	232	134	140	302	302	204	246	158	158	344	348	142	142	276	276	236	236	160	172
579	A007	Wild.cult	232	232	140	140	292	292	246	246	158	158	348	348	142	142	276	276	236	236	172	172
580	A007	Wild.cult	232	242	134	150	292	292	204	212	158	160	344	348	130	142	274	276	236	236	158	172
581	A007	Wild.cult	232	232	134	140	292	292	204	204	158	158	344	348	142	142	276	276	236	236	172	172
582	A007	Wild.cult	232	232	140	140	292	292	204	204	158	158	344	344	132	142	276	276	236	236	172	172
583	A007	Wild.cult	232	232	140	140	292	292	246	246	158	158	344	348	142	142	276	276	236	236	172	172
584	A007	Wild.cult	232	242	134	150	292	292	204	212	0	0	344	344	142	146	276	276	236	236	158	172
585	A007	Wild.cult	232	242	134	150	292	292	204	212	158	160	344	344	130	142	276	276	236	236	170	172
586	A007	Cult	232	242	140	150	292	292	204	212	158	160	344	344	130	142	276	276	236	260	158	172
587	A007	Wild.cult	232	232	134	134	292	292	204	246	158	158	344	348	142	142	276	276	236	236	160	172
588	A007	Wild.cult	232	232	140	140	292	292	246	246	158	158	348	348	142	142	276	276	236	236	172	172
223	A008	Wild.cult	232	232	140	140	292	292	212	246	158	158	344	344	142	142	276	276	236	236	170	170
224	A008	Wild.cult	232	232	140	140	292	302	212	246	158	158	344	348	142	142	276	276	236	236	170	172
225	A008	Wild.cult	232	232	140	140	292	292	194	220	158	158	348	348	142	142	276	276	236	236	170	172
226	A008	Wild.cult	232	232	140	140	292	302	212	212	158	158	344	348	142	142	276	276	236	236	170	170
589	A008	Wild.cult	232	232	140	140	292	302	212	246	158	158	344	344	142	142	276	276	236	236	170	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
590	A008	Wild.cult	232	232	140	140	292	292	212	246	158	158	348	348	142	142	276	276	236	236	170	172
591	A008	Wild.cult	232	242	138	140	292	292	212	212	158	160	344	344	142	142	274	274	236	238	158	170
592	A008	Wild.cult	232	242	138	140	292	318	206	246	158	160	338	344	130	142	272	276	236	236	158	170
593	A008	Wild.cult	232	242	138	140	302	318	212	246	158	160	344	348	130	142	276	276	236	238	158	172
594	A008	Wild.cult	228	232	138	140	302	302	206	246	158	160	338	344	130	142	272	276	236	236	146	172
595	A008	Wild.cult	228	232	138	140	292	318	206	246	158	160	338	344	142	142	272	276	236	236	146	172
596	A008	Wild.cult	228	232	138	140	292	318	212	246	158	160	344	348	142	142	274	276	236	238	158	172
597	A008	Wild.cult	228	232	138	140	292	292	206	246	158	160	344	344	130	142	274	276	236	236	146	172
598	A008	Wild.cult	228	232	138	140	302	318	206	246	158	160	338	344	130	142	272	276	236	236	146	170
195	A013	Cult	232	242	134	138	292	318	212	236	160	162	344	344	130	142	274	274	0	0	0	0
227	A013	Cult	232	242	134	156	292	318	212	236	160	162	336	344	130	142	274	274	236	236	148	170
228	A013	Cult	232	232	134	134	292	292	212	246	158	160	344	348	130	142	276	276	236	258	160	170
230	A014	Cult	228	250	138	154	318	318	190	202	156	160	336	346	130	142	274	284	238	240	146	148
231	A016	Cult	0	0	156	156	306	306	212	214	160	160	344	344	130	142	274	274	236	240	148	158
232	A016	Cult	250	228	156	156	306	306	212	212	160	162	344	344	142	142	274	274	236	240	148	158
599	A016	Cult	228	232	138	156	0	0	206	220	160	160	338	344	130	130	272	276	236	238	148	170
600	A016	Cult	232	232	138	138	292	318	212	220	156	160	344	344	130	142	274	276	236	238	146	158
601	A016	Cult	228	242	134	138	0	0	212	212	160	160	344	344	130	130	274	274	236	236	148	170
602	A016	Cult	228	242	138	138	292	292	206	220	160	160	338	344	130	142	272	276	236	238	146	158
603	A016	Cult	228	232	134	156	292	2 92	212	212	160	160	344	344	130	142	274	274	236	236	148	158

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
604	A016	Cult	228	242	134	138	318	318	206	212	160	160	338	344	130	142	272	274	236	238	148	158
605	A016	Cult	232	242	134	138	292	292	206	220	156	160	338	344	130	142	274	276	236	236	148	158
606	A016	Cult	228	242	138	138	0	0	206	220	156	160	338	344	130	130	272	274	236	238	146	170
607	A016	Cult	232	242	134	138	0	0	206	220	160	160	336	344	130	130	272	276	236	236	148	170
608	A016	Cult	232	242	138	138	292	318	212	220	156	160	344	344	130	130	274	274	236	236	146	170
233	A017	Cult	228	228	156	156	306	306	202	206	160	160	336	338	130	142	272	274	238	240	158	158
234	A017	Cult	228	228	138	156	306	306	212	212	160	160	344	344	130	142	274	274	236	238	146	158
235	A018	Cult	232	232	138	140	292	318	212	246	160	162	338	344	130	146	272	276	236	236	146	170
236	A018	Cult	232	232	138	140	292	318	206	246	160	160	336	344	130	142	274	276	236	238	154	170
237	A018	Cult	232	242	138	140	292	318	206	246	160	160	338	344	142	146	272	276	236	238	158	170
609	A019	Cult	232	232	140	140	292	292	212	236	160	160	348	348	142	146	276	276	236	236	170	170
610	A019	Cult	232	232	134	140	290	290	236	236	160	160	344	344	142	146	276	276	236	236	170	170
611	A019	Cult	232	232	134	134	292	292	212	212	160	160	348	348	142	146	276	276	236	236	170	170
612	A019	Cult	232	232	134	134	290	290	212	236	160	160	344	348	142	146	276	276	236	236	170	170
613	A019	Cult	232	232	134	140	292	292	212	236	160	160	348	348	142	142	276	276	236	236	170	170
614	A019	Cult	232	232	134	140	290	292	212	236	160	160	344	348	146	146	276	276	236	236	170	170
615	A019	Cult	232	232	140	140	290	292	212	236	160	160	348	348	146	146	276	276	236	236	170	170
616	A019	Cult	232	232	134	140	290	292	212	236	160	160	344	348	146	146	276	276	236	236	170	170
617	A019	Cult	232	232	134	140	290	290	212	212	160	160	348	348	142	146	276	276	236	236	170	170
618	A019	Cult	232	232	134	140	290	292	212	212	160	160	348	348	142	142	276	276	236	236	170	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
244	A024	Cult	0	0	134	150	292	292	206	220	160	160	344	344	130	146	274	274	254	260	148	170
246	A024	Cult	232	232	134	150	292	292	206	212	156	162	334	344	130	150	276	284	254	258	148	170
619	A024	Cult	232	232	134	134	292	292	212	246	158	162	344	348	130	142	276	276	236	238	170	170
620	A024	Cult	232	232	134	140	292	292	212	246	158	160	344	348	130	146	276	276	236	236	170	170
621	A024	Cult	232	232	134	138	292	292	212	246	160	160	344	348	130	142	274	274	236	236	148	170
622	A024	Cult	232	232	138	140	292	292	212	246	158	162	344	348	142	146	276	276	236	238	148	170
623	A024	Cult	232	232	134	140	292	292	212	246	160	160	344	348	146	146	276	276	236	238	170	170
624	A024	Cult	232	232	134	134	292	292	212	246	158	160	344	344	142	146	274	274	236	238	148	170
625	A024	Cult	232	232	134	138	292	292	212	246	158	162	344	344	130	146	276	276	236	238	170	170
626	A024	Cult	232	232	134	140	292	292	212	246	158	160	344	348	130	146	276	276	236	236	170	170
627	A024	Cult	232	232	134	138	292	292	212	246	160	160	344	344	146	146	276	276	236	236	148	170
628	A024	Cult	232	232	134	138	292	292	212	246	160	160	344	348	142	146	276	276	236	238	170	170
248	A025	Cult	232	242	134	136	292	304	204	220	160	162	346	356	130	142	274	274	236	238	158	170
250	A025	Cult	232	242	134	136	292	304	204	220	160	162	346	356	142	142	274	274	236	238	158	170
251	A025	Cult	232	242	134	136	292	304	204	220	156	162	346	356	142	142	274	274	236	238	170	176
252	A025	Cult	232	242	134	136	292	304	204	220	160	162	346	356	130	146	274	274	236	238	158	170
629	A026	Cult	232	232	138	138	292	292	206	246	160	162	338	344	142	142	272	274	242	242	146	146
630	A026	Cult	232	232	138	138	292	318	206	246	160	162	338	344	142	146	272	276	236	236	146	170
631	A031	Cult	232	232	138	138	292	318	212	212	162	162	336	344	142	146	274	276	236	238	170	170
632	A031	Cult	232	242	138	138	318	318	214	220	0	0	336	344	142	146	274	276	236	238	160	160

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
633	A031	Cult	232	242	138	138	292	292	220	220	0	0	336	344	146	146	274	276	236	238	160	170
634	A031	Cult	232	232	138	140	318	318	214	220	162	162	336	344	146	146	276	276	238	238	170	170
635	A031	Cult	242	242	138	140	292	318	214	220	162	162	336	344	146	146	274	276	236	236	160	160
636	A031	Cult	240	242	138	140	292	292	206	214	156	160	334	336	130	146	274	274	236	238	170	176
172	A032	Cult	232	232	140	140	292	292	220	220	162	162	344	344	142	142	276	276	234	236	170	170
147	A033	Cult	232	232	136	136	318	318	212	216	160	160	344	344	130	130	276	276	238	238	148	152
148	A033	Cult	228	232	136	136	318	318	212	216	160	160	344	344	130	130	276	276	238	238	148	152
149	A033	Cult	228	232	136	136	310	310	212	216	160	160	344	344	130	130	276	276	238	238	148	152
150	A033	Cult	228	232	136	150	310	318	216	216	160	160	344	344	130	130	276	276	238	238	148	148
151	A033	Cult	228	232	136	136	310	318	216	216	160	160	344	344	130	130	276	276	238	238	148	152
158	A033	Cult	228	228	150	150	318	318	216	216	160	160	344	344	130	130	274	274	238	238	148	148
159	A033	Cult	228	228	150	150	310	318	212	216	160	160	344	344	130	130	274	274	238	238	152	152
160	A033	Cult	228	232	136	150	310	318	216	216	160	160	344	344	130	130	276	276	238	238	152	152
177	A034	Cult	232	232	140	140	292	292	214	216	166	166	344	346	142	142	274	276	236	238	168	170
178	A034	Cult	232	232	138	138	292	292	214	214	156	162	344	346	146	146	274	276	238	238	170	170
179	A034	Cult	232	232	134	140	290	292	202	204	156	160	336	344	146	146	274	276	236	236	170	170
181	A034	Cult	232	232	126	134	292	292	204	246	162	162	344	344	146	146	276	276	236	236	170	170
182	A034	Cult	232	232	138	140	292	292	212	214	156	162	344	346	142	146	274	276	236	236	170	170
183	A034	Cult	232	232	134	134	292	292	204	204	162	162	344	344	146	146	276	276	236	236	170	170
184	A034	Cult	232	232	138	140	292	292	214	214	156	156	344	344	142	146	276	276	236	238	168	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
637	A034	Cult	232	232	134	134	292	292	190	212	156	162	344	346	146	146	274	276	236	236	168	170
638	A034	Cult	232	232	150	150	292	292	190	212	156	162	344	346	130	146	274	276	234	234	168	168
639	A034	Cult	228	232	134	150	292	292	190	212	156	162	346	346	130	146	274	274	236	236	170	170
640	A034	Cult	228	228	134	150	0	0	212	212	162	162	344	344	130	146	276	276	234	236	168	170
152	A036	Cult	232	242	134	140	292	292	214	220	156	156	336	344	146	146	274	276	236	238	170	170
153	A036	Cult	232	234	134	150	292	292	246	246	156	160	344	344	130	146	276	276	236	236	158	170
154	A036	Cult	232	242	138	140	292	318	214	220	162	162	336	344	142	146	274	276	236	238	170	170
155	A036	Cult	232	232	134	140	292	304	204	246	156	162	344	344	130	146	276	276	236	236	170	170
157	A036	Cult	232	234	134	150	290	292	202	204	156	160	330	344	130	146	274	276	236	236	158	170
161	A036	Cult	232	234	134	150	290	292	202	246	156	160	344	344	130	146	276	276	236	242	158	170
641	A036	Cult	232	232	140	140	292	292	220	220	0	0	344	344	142	146	276	276	236	236	170	170
642	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	142	142	276	276	234	234	170	170
643	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	146	146	276	276	234	234	170	170
644	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	142	142	276	276	234	236	170	170
645	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	142	146	276	276	234	234	170	170
646	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	142	146	276	276	234	236	170	170
647	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	142	146	276	276	234	234	170	170
648	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	146	146	276	276	234	236	170	170
649	A036	Cult	232	232	140	140	292	292	220	220	162	162	344	344	142	146	276	276	234	234	170	170
167	A040	Cult	228	228	134	150	292	292	190	212	156	156	344	346	146	146	274	276	234	236	168	168

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
168	A040	Cult	232	234	134	140	292	292	204	246	162	162	344	344	146	146	276	276	236	236	158	170
173	A040	Cult	232	232	138	138	292	292	212	214	156	162	344	346	142	146	274	276	236	238	168	170
174	A040	Cult	232	232	140	140	292	292	212	214	156	158	344	346	142	142	276	276	236	238	168	168
175	A040	Cult	232	232	138	140	292	292	212	212	156	156	344	344	142	146	276	276	236	238	168	168
176	A040	Cult	232	232	138	140	292	292	212	212	152	156	344	346	142	142	274	276	236	238	168	170
180	A040	Cult	232	232	138	140	292	292	212	214	156	156	344	346	146	146	274	276	236	238	168	168
650	A040	Cult	232	232	138	140	292	292	212	214	156	156	344	346	142	146	274	276	238	238	170	170
651	A040	Cult	232	232	138	138	0	0	212	214	156	162	344	346	146	146	274	276	236	236	168	170
652	A040	Cult	232	232	138	138	292	292	212	214	156	156	344	344	146	146	276	276	236	238	168	168
653	A040	Cult	232	232	138	140	292	292	212	214	156	162	344	346	142	142	274	276	236	238	168	170
654	A040	Cult	232	232	138	140	292	292	212	212	156	156	346	346	142	146	274	274	236	238	170	170
655	A040	Cult	232	232	138	140	0	0	212	214	156	162	344	346	142	146	274	276	236	236	168	168
656	A040	Cult	232	232	140	140	292	292	212	214	156	162	344	346	146	146	274	276	236	238	170	170
657	A040	Cult	232	232	140	140	292	292	212	214	162	162	344	344	142	146	276	276	236	236	168	170
658	A040	Cult	232	232	138	140	292	292	212	212	156	156	344	344	142	146	276	276	238	238	170	170
659	A040	Cult	232	232	138	140	292	292	214	214	156	156	344	346	146	146	274	276	238	238	168	170
132	A042	Cult	250	250	150	150	304	304	202	214	156	156	336	346	142	142	274	274	236	238	146	148
133	A042	Cult	232	234	150	150	304	304	214	214	156	156	346	346	142	142	274	274	236	238	146	158
136	A042	Cult	232	234	150	156	304	304	202	214	160	160	336	344	130	142	274	274	236	238	146	158
137	A042	Cult	250	250	150	156	304	304	214	214	160	160	346	346	130	142	274	274	236	238	158	158

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
660	A042	Cult	228	232	138	150	318	318	190	212	156	156	344	346	130	130	274	274	234	236	146	148
661	A042	Cult	232	232	138	150	318	318	190	212	156	156	344	346	130	130	274	274	236	236	148	158
662	A042	Cult	228	232	150	150	304	318	212	214	156	160	344	346	130	142	274	274	238	254	158	168
663	A042	Cult	228	232	138	150	0	0	190	212	156	156	344	346	130	142	274	274	236	236	146	148
664	A042	Cult	232	232	138	138	304	318	190	214	156	156	346	346	130	130	274	274	236	254	148	158
121	A043	Cult	250	250	150	150	306	318	202	202	156	160	336	346	150	150	270	270	238	238	148	148
122	A043	Cult	234	242	150	150	304	318	214	214	156	160	346	346	142	142	274	274	238	242	158	158
123	A043	Cult	242	250	150	150	306	318	202	202	156	156	336	338	142	142	272	274	238	242	148	148
125	A043	Cult	234	242	138	150	304	318	202	202	156	156	336	338	142	142	272	274	242	242	148	148
126	A043	Cult	234	242	138	150	304	318	202	214	156	160	336	338	142	142	272	274	238	238	148	158
127	A043	Cult	234	242	150	150	304	318	214	214	156	160	338	346	142	142	272	274	238	238	148	148
128	A043	Cult	250	250	138	150	306	318	202	214	156	160	336	338	142	142	272	274	238	242	148	148
129	A043	Cult	250	250	150	150	304	318	214	214	156	160	336	346	142	142	274	274	238	238	158	158
131	A044	Cult	250	250	138	150	306	306	190	190	156	160	334	344	142	142	274	284	256	256	158	168
290	A044	Cult	250	250	138	150	318	318	190	202	156	160	334	346	130	142	274	274	236	256	158	168
291	A044	Cult	232	250	138	150	306	318	202	212	160	160	338	344	130	142	274	274	236	238	158	168
292	A044	Cult	250	250	150	150	318	318	190	202	160	160	338	346	142	142	274	274	238	256	146	158
665	A044	Cult	232	234	134	150	292	306	190	212	156	162	334	344	130	146	276	284	236	238	158	170
666	A044	Cult	232	250	134	150	292	306	190	212	156	160	334	344	130	146	274	284	236	238	158	170
667	A044	Cult	232	234	134	150	292	306	190	212	160	160	334	344	130	146	276	284	236	238	158	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
668	A044	Cult	232	250	134	150	292	318	202	220	160	160	338	344	142	146	274	276	236	238	158	170
669	A044	Cult	232	234	134	150	292	318	202	212	160	162	338	344	130	146	274	274	236	256	158	170
670	A044	Cult	232	234	134	150	292	306	202	212	160	160	334	344	130	146	274	284	236	256	158	170
671	A044	Cult	232	250	134	150	292	318	190	220	160	162	338	344	130	146	276	284	236	256	158	170
672	A044	Cult	232	234	134	150	292	306	202	220	156	160	338	344	142	146	274	276	236	256	158	170
673	A044	Cult	232	250	134	150	292	306	190	212	160	160	334	344	142	146	274	284	236	238	158	170
674	A044	Cult	232	234	134	150	292	306	202	212	160	162	338	344	130	146	274	274	236	238	158	170
138	A048	Cult	232	242	138	138	290	318	214	246	156	160	334	344	142	150	276	284	238	256	158	168
139	A048	Cult	232	242	138	140	304	318	190	206	156	162	334	346	142	150	274	274	236	238	148	170
140	A048	Cult	242	242	138	140	290	304	206	246	160	162	334	344	142	150	276	276	236	238	148	170
141	A048	Cult	232	250	138	138	290	304	190	206	160	164	334	346	142	142	274	274	236	238	170	170
142	A048	Cult	242	250	138	150	304	318	206	246	156	156	334	344	142	142	274	276	236	256	148	158
144	A048	Cult	242	250	138	150	304	318	214	246	160	162	334	344	142	150	276	284	238	256	170	170
675	A048	Cult	232	232	138	150	304	318	190	212	156	156	334	344	130	142	274	284	238	238	148	168
676	A048	Cult	232	242	150	150	304	318	190	212	156	160	334	344	142	142	274	284	236	238	148	148
677	A048	Cult	232	242	150	150	318	318	190	214	156	160	334	344	130	142	274	284	238	238	148	168
134	A049	Cult	232	232	150	150	318	318	212	214	160	160	344	346	130	142	274	274	238	256	158	168
135	A049	Cult	250	250	150	150	318	318	190	212	156	160	344	346	130	142	274	274	236	236	158	168
143	A049	Cult	228	232	138	150	304	318	190	214	156	160	346	346	130	142	274	274	236	256	158	168
145	A049	Cult	232	250	138	138	0	0	190	214	156	156	344	346	142	146	274	274	236	236	146	148

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
146	A049	Cult	232	250	150	150	318	318	212	214	160	160	344	344	130	142	274	274	236	256	146	148
263	A049	Cult	232	232	150	150	296	296	198	200	160	160	344	346	130	142	274	274	238	254	158	168
130	A050	Cult	234	242	138	150	304	318	202	214	156	156	338	346	130	142	274	284	238	242	158	158
99	A051	Wild	232	232	134	134	292	292	212	220	160	160	0	0	142	142	276	276	236	260	170	170
100	A051	Wild	232	232	134	134	290	292	212	220	160	160	344	344	142	142	276	276	236	260	170	170
264	A051	Wild	232	232	134	134	292	292	212	220	160	160	344	344	142	142	276	276	236	258	170	170
265	A051	Wild	232	232	134	134	292	292	212	220	160	160	344	344	142	142	276	276	258	258	170	170
266	A051	Wild	232	232	140	140	292	292	212	220	160	160	344	344	142	142	276	276	236	236	170	170
678	A060	Wild	232	232	134	140	290	292	212	246	158	160	344	344	142	142	276	276	236	236	170	170
679	A060	Wild	232	232	134	140	290	292	212	246	158	160	344	344	142	142	276	276	236	236	170	170
680	A060	Wild	232	232	140	140	292	292	212	212	158	160	344	344	142	142	276	276	236	236	170	170
681	A060	Wild	0	0	140	140	290	292	212	246	0	0	344	344	142	142	276	276	236	236	170	170
682	A060	Wild	232	232	140	140	292	292	212	246	160	160	344	344	142	142	276	276	236	236	170	170
683	A060	Wild	232	232	134	140	290	292	212	212	160	160	344	344	142	142	276	276	236	236	170	170
684	A060	Wild	232	232	140	140	292	292	246	246	162	162	344	344	142	142	276	276	236	236	170	170
685	A061	Wild	232	232	134	140	290	292	212	212	158	162	344	344	142	142	276	276	236	236	170	170
686	A061	Wild	232	232	134	140	292	292	246	246	160	162	350	350	142	142	276	276	236	236	170	170
687	A061	Wild	232	232	134	140	292	292	212	212	158	162	344	344	142	142	276	276	236	236	170	170
688	A061	Wild	232	232	140	140	292	292	212	212	158	162	344	344	142	142	276	276	236	236	170	170
689	A061	Wild	232	232	140	140	292	292	212	246	160	162	344	344	142	142	276	276	236	236	170	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
690	A061	Wild	232	232	134	140	290	292	212	212	158	158	344	344	142	142	276	276	236	236	170	170
691	A061	Wild	232	232	134	140	290	292	212	246	160	160	344	344	142	142	276	276	236	236	170	170
692	A061	Wild	232	232	140	140	292	292	212	246	148	160	344	344	142	142	276	276	236	236	170	170
693	A061	Wild	232	232	140	140	292	292	246	246	158	160	344	344	142	142	276	276	236	236	170	170
694	A061	Wild	232	232	140	140	290	292	212	212	158	162	344	344	142	142	276	276	236	236	170	170
96	A062	Wild	232	232	140	140	292	310	220	246	158	160	344	344	142	146	276	276	236	236	170	170
97	A062	Wild	232	232	134	134	292	310	212	220	160	162	344	348	142	142	276	276	236	236	160	170
98	A062	Wild	232	232	134	134	290	294	220	246	160	160	344	348	142	146	274	276	236	236	170	170
267	A062	Wild	232	232	134	134	290	292	212	236	160	160	344	348	142	146	276	276	236	236	170	170
268	A062	Wild	232	232	134	134	294	310	212	236	160	160	344	348	142	142	276	276	236	236	170	170
92	A066	Wild	232	232	134	140	290	292	212	220	160	160	344	344	142	146	276	276	236	236	170	170
94	A066	Wild	232	232	134	148	292	310	204	220	158	160	344	344	142	142	276	276	236	254	170	170
269	A066	Wild	232	232	140	148	292	310	204	220	158	160	344	344	142	146	276	276	236	254	170	170
270	A066	Wild	232	232	140	140	292	292	220	220	160	160	344	344	146	146	276	276	254	258	170	170
271	A066	Wild	232	232	134	134	290	292	220	246	160	162	344	344	142	146	276	276	236	236	0	0
272	A066	Wild	232	232	134	140	290	292	220	246	160	162	344	344	146	146	276	276	234	234	170	170
273	A066	Wild	232	232	134	134	290	292	220	246	160	160	344	344	142	150	276	276	236	236	160	170
91	A067	Wild	232	232	134	140	290	292	220	246	158	160	344	344	142	146	276	276	236	236	170	170
93	A067	Wild	232	232	134	134	292	292	212	212	160	162	344	348	146	150	276	276	236	236	170	170
95	A067	Wild	232	232	134	136	290	292	212	220	160	160	344	344	146	146	276	276	236	260	160	170

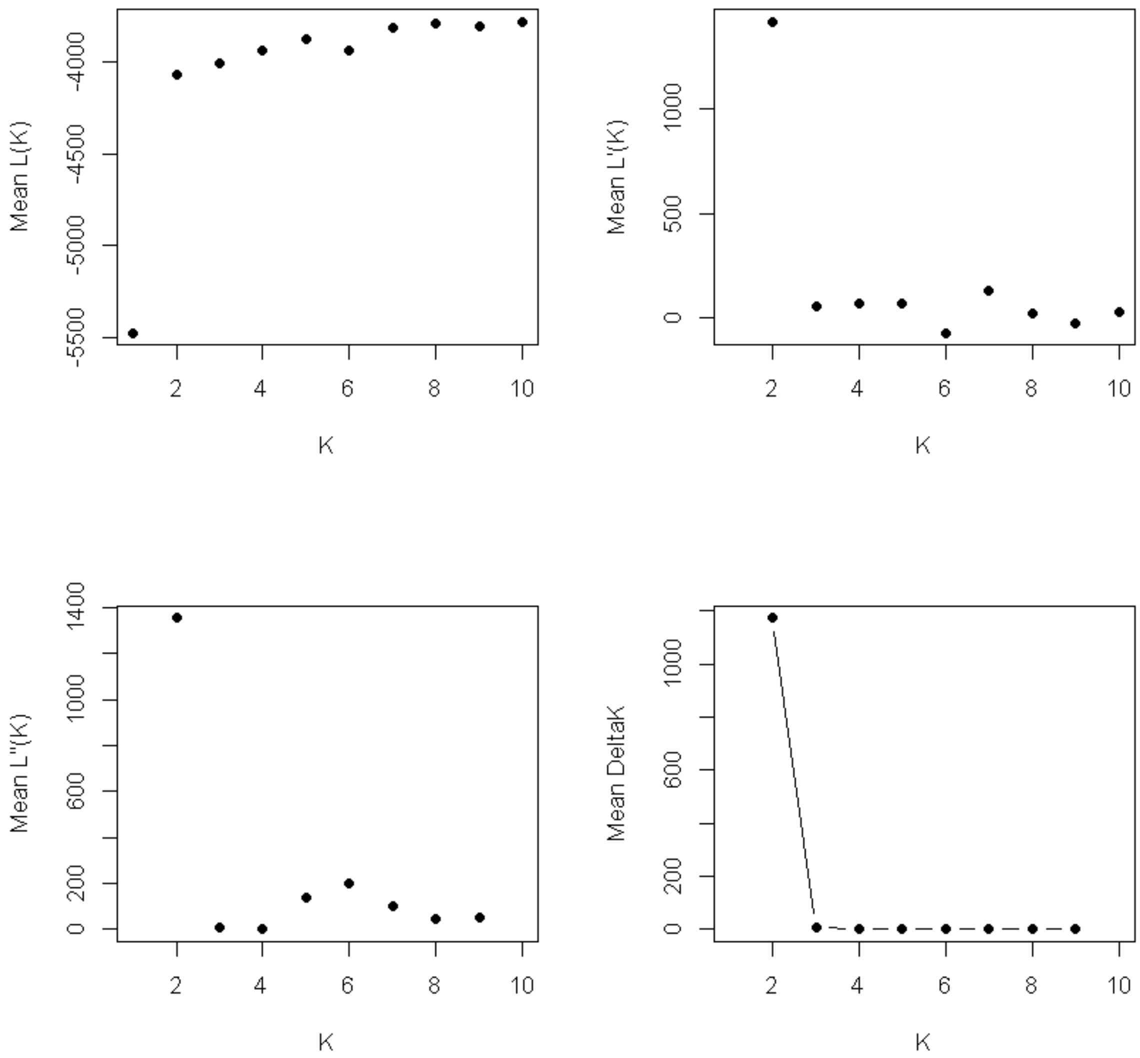
ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
274	A067	Wild	232	232	134	134	290	292	212	246	160	162	344	344	142	146	276	276	236	236	160	170
275	A067	Wild	232	232	134	134	290	292	220	246	160	160	344	348	146	146	276	276	236	236	170	170
276	A067	Wild	232	232	134	136	290	292	212	220	160	160	344	344	146	146	276	276	236	258	160	170
277	A067	Wild	232	232	134	134	290	292	212	220	160	160	344	348	142	146	276	276	236	236	160	170
278	A067	Wild	232	232	134	134	292	292	212	212	160	162	344	348	146	150	276	276	236	236	170	170
103	A072	Wild	232	232	134	134	292	292	220	246	160	160	344	344	142	146	276	276	236	236	170	170
104	A072	Wild	232	232	134	134	292	292	220	236	160	160	344	344	146	150	276	276	236	236	170	170
105	A072	Wild	232	232	134	140	292	292	216	236	160	160	344	350	142	150	276	276	236	236	170	170
106	A072	Wild	232	232	134	140	292	292	216	246	160	160	344	348	146	150	276	276	236	236	170	170
107	A072	Wild	232	232	134	134	292	292	216	246	160	160	344	344	142	146	276	276	236	236	170	170
119	A072	Wild	232	232	140	140	290	292	220	236	160	162	344	344	142	142	276	276	236	236	170	172
279	A072	Wild	232	232	134	134	292	292	190	212	160	160	344	344	142	146	276	276	236	236	170	170
280	A072	Wild	232	232	134	140	292	292	218	236	160	160	344	348	142	150	276	276	236	236	170	170
695	A075	Wild	232	232	134	140	292	292	204	220	158	158	344	344	146	146	276	276	234	236	170	170
696	A075	Wild	232	232	134	140	292	292	204	236	160	162	344	348	142	146	272	272	234	236	170	170
697	A075	Wild	232	232	134	140	292	310	204	236	160	162	344	348	142	142	276	276	234	236	170	170
698	A075	Wild	232	232	134	140	292	310	212	236	158	160	344	348	146	146	276	276	236	236	170	170
699	A075	Wild	232	232	134	134	292	292	212	236	158	158	344	344	142	142	276	276	236	236	170	170
700	A075	Wild	232	232	134	140	292	292	212	236	160	162	344	344	142	146	276	276	236	236	170	170
701	A075	Wild	232	232	134	134	292	310	204	220	160	162	344	348	142	146	276	276	234	236	170	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
702	A075	Wild	232	232	134	134	292	310	212	236	158	160	344	344	142	146	276	276	236	236	170	170
703	A075	Wild	232	232	140	140	292	310	204	204	162	162	344	348	142	142	276	276	234	236	170	170
101	A076	Wild	232	232	134	134	290	292	220	246	162	162	344	344	142	146	276	276	236	236	170	170
102	A076	Wild	232	232	134	134	290	292	220	246	160	160	344	348	142	146	276	276	236	236	170	170
108	A076	Wild	232	232	134	134	292	292	212	220	160	162	344	344	142	146	276	276	236	236	170	170
109	A076	Wild	232	232	134	134	292	310	236	246	160	160	344	348	142	146	276	276	236	236	170	170
110	A076	Wild	232	232	134	134	290	290	220	246	160	162	344	348	146	146	276	276	236	236	170	170
281	A076	Wild	232	232	134	134	290	290	220	246	162	162	344	348	142	142	276	276	236	236	170	170
282	A076	Wild	232	232	134	134	292	310	236	246	160	160	344	348	142	142	276	276	236	236	170	170
283	A076	Wild	232	232	134	134	290	292	220	246	160	162	344	348	142	146	276	276	236	236	170	170
284	A076	Wild	232	232	130	134	292	292	220	246	162	162	344	344	142	142	276	276	236	236	170	170
285	A076	Wild	232	232	134	134	292	292	204	220	160	160	344	348	142	146	276	276	236	236	170	170
286	A076	Wild	232	232	134	134	290	292	220	246	162	162	344	348	142	146	276	276	236	236	170	170
287	A076	Wild	232	232	134	134	292	292	220	246	162	162	348	348	142	146	276	276	236	236	170	170
111	A079	Wild	232	232	134	134	290	290	220	220	160	160	344	348	146	150	276	276	236	236	170	170
112	A079	Wild	232	232	134	134	290	290	220	220	160	160	344	348	142	150	276	276	236	236	170	170
113	A079	Wild	232	232	134	140	290	292	220	220	160	162	344	344	142	142	276	276	236	236	170	170
114	A079	Wild	232	232	134	140	292	310	220	220	160	162	344	344	142	146	276	276	236	236	170	170
115	A079	Wild	232	232	134	140	290	292	236	246	162	162	344	348	142	146	276	276	236	236	170	170
116	A079	Wild	232	232	134	134	290	292	220	220	160	160	344	348	146	150	276	276	236	236	170	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
117	A079	Wild	232	232	134	140	292	292	220	246	160	160	344	348	142	142	276	276	236	236	170	170
118	A079	Wild	232	232	134	140	290	292	220	246	160	162	344	344	142	146	276	276	236	236	170	172
120	A079	Wild	232	232	134	134	290	290	220	246	160	162	344	348	142	146	276	276	236	236	170	170
208	A079	Wild	232	232	134	134	290	292	220	246	160	162	346	350	0	0	0	0	0	0	0	0
288	A079	Wild	232	232	134	140	290	292	236	246	160	162	344	344	142	142	276	276	236	236	170	170
289	A079	Wild	232	232	134	140	290	292	236	246	162	162	344	348	142	146	276	276	236	236	170	170
704	A084	Wild	232	232	134	134	290	292	220	246	160	162	344	344	146	146	276	276	236	236	170	170
705	A084	Wild	232	232	134	134	292	292	220	220	160	160	344	344	142	146	276	276	236	236	170	170
706	A084	Wild	232	232	134	134	292	292	220	246	160	162	344	348	142	146	276	276	236	236	170	170
707	A084	Wild	232	232	134	134	290	292	220	220	160	160	344	348	142	146	276	276	236	236	170	170
708	A084	Wild	232	232	134	134	290	292	220	246	160	162	344	344	146	146	276	276	236	236	170	170
709	A084	Wild	232	232	134	134	292	292	220	246	162	162	348	348	146	146	276	276	236	236	170	170
710	A084	Wild	232	232	134	134	290	290	246	246	162	162	344	348	142	146	276	276	236	236	170	170
711	A084	Wild	232	232	134	134	292	292	220	220	160	162	344	348	142	142	276	276	236	236	170	170
712	A088	Wild	0	0	134	140	290	292	212	246	160	160	344	344	142	142	276	276	236	236	170	170
713	A088	Wild	232	232	140	140	292	292	212	246	158	160	344	344	142	142	276	276	236	236	170	170
714	A088	Wild	232	232	140	140	292	292	212	246	158	160	344	344	142	142	276	276	236	236	170	170
715	A088	Wild	232	232	134	140	292	292	212	212	160	162	344	344	142	142	276	276	236	236	170	170
716	A088	Wild	232	232	140	140	292	292	246	246	158	162	344	344	142	142	276	276	236	236	170	170
717	A088	Wild	232	232	140	140	292	292	212	246	160	160	344	344	142	142	276	276	236	236	170	170

ID	Mother tree	Type	MTcCir6		MTcCir25		MTcCir11		MTcCir12		MTcCir7		MTcCir18		MTcCir1		MTcCir17		MTcCir15		MTcCir21	
718	A088	Wild	232	232	140	140	292	292	246	246	160	160	344	344	142	142	276	276	236	236	170	170
719	A088	Wild	232	232	140	140	292	292	246	246	160	160	344	344	142	142	276	276	236	236	170	170
720	A088	Wild	232	232	134	140	290	292	212	212	158	162	344	344	142	142	276	276	236	236	170	170
721	A088	Wild	232	232	140	140	292	292	246	246	160	162	344	344	142	142	276	276	236	236	170	170

Note. Cult = cultivated; Cult/h = putative hybrids; Wild.cult = cultivated wild cacao



Appendix 2.6 Analysis of the most probable number of clusters, *K*, according to Evanno *et al*. (2005). For each *K* (1..10), 10 Structure runs were summarized. The existence of two clusters is indicated by a maximum of mean D*K* at *K* = 2.

CHAPTER III

FLORAL VISITOR DIVERSITY AND ABUNDANCE ON WILD AND CULTIVATED CACAO (*THEOBROMA CACAO* L.) IN BOLIVIA

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(Manuscript to be submitted to *Agroforestry Systems*)

ABSTRACT

Despite the economic importance of *T. cacao*, amazingly little is known about its pollination ecology. Ceratopogonid midges are considered the main pollinators, but limited evidence on the sexual reproduction is based almost exclusively on cultivated cacao and knowledge is nonexistent for wild populations. Indeed, many other potentially pollinating insects are attracted to *T. cacao* flowers, possibly seeking nectar or pollen. In view of the numerous uncertainties surrounding the pollination ecology of *T. cacao*, in our study we documented flower visitors in wild and cultivated plants of *T. cacao.* We put glue on 2237 flowers of wild and cultivated cacao trees in Bolivia to trap floral visitors and found 631 insects belonging to eight orders. The capture rate was 0.3 insects per flower on wild and cultivated cacao. The most abundant and diverse insect order on both cacao types was Hymenoptera represented mainly by small parasitoids. Hymenoptera were more abundant on wild cacao, however species richness was higher in cultivated cacao. The relative abundance and species richness of Diptera were not significantly different between wild and cultivated cacao. However, species composition and proportion of Diptera species differed between both wild and cultivated cacao. The most abundant families of Diptera were Chloropidae and Phoridae. Ceratopogonidae were only represented by 13 individuals belonging to 7 species. Thysanoptera and Hemiptera were significantly more abundant on cultivated cacao flowers. Only a single specimen collected carried cacao pollen and we were thus unable to identify actual pollinators. In any case, we found significant differences among the visitor assemblages between wild and cultivated cacao, which suggest that midges alone were probably too rare to act as main or even sole pollinators of cacao in our study region.

Potential additional pollinators would be small Diptera (e.g., Chloropidae and Phoridae) and Hymenoptera (e.g., Eulophidae and Platygastridae).

Key words: Pollinator, Diptera, Hymenoptera, *Forcypomyia*, Bolivia, midge.

INTRODUCTION

Cacao (*Theobroma cacao* L., Malvaceae) is one of the world's most important cash crop species. Yet despite its economic importance, surprisingly little is known about the pollination ecology of the species. Cacao is generally considered to be mainly pollinated by tiny midges (Ceratopogonidae) but knowledge on the sexual reproduction is based almost exclusively on limited evidence from cultivated cacao, often from parts of the non-native range of the species (Wellensiek, 1932; Soria, 1973; Soria and Wirth 1974; Soria *et al.*, 1980), and is nonexistent for wild populations.

It has been suggested that the tiny ceratopogonid midges *Forcipomyia blantoni* Soria and Bystrak (Soria *et al.*, 1980), *Forcipomyia youngi* Wirth and *F. quatei* Wirth (Young, 1983) are the most effective cacao pollinators. Nevertheless, cacao pollinator surveys have often found low abundances of ceratopogonid midges (Entwistle, 1972; Young 1986; J. L. Groenevald pers. comm.). Indeed, many other potentially pollinating insects are attracted to cacao flowers, possibly seeking nectar or pollen. Thus, in addition to ceratopogonid midges, members of other Dipterian families such as Phoridae, Sciaridae and Drosophilidae have been captured on cacao flowers (Young, 1986). Young (1987) found mainly thrips (70%) and aphids on cacao flowers, and only one ceratopogonid individual with pollen. Cecidomyiid midges have also been found carrying cacao pollen grains (Lucas, 1981). Stingless bees are further occasional floral visitors of cacao (Soria, 1975; Young, 1981). Thus, although ceratopogonid midges are considered the main pollinator agents, evidence in this regard is actually rather contradictory and one mayor open question regards the flower visitors of non-cultivated cacao plants in their natural habitat.

Which floral rewards attract pollinators to *T. cacao* is unclear (Wellensiek, 1932; Soria *et al*., 1980; Young *et al*., 1984). Unique floral structures in *Theobroma* suggest highly specialized pollination adaptations for pollination by flying insects (Young *et al*., 1987). *Theobroma cacao* flowers have nectaries on the pedicels, sepals, ovaries, "guide lines" of petals and staminodia (Stejskal, 1969), and are fragrant (Young *et al*., 1984). The brightly coloured spatule ligule of the flowers could play a role in pollinator attraction (Young *et al*., 1984). The flowers also have ultraviolet light reflectance/absorbance, which probably attract pollinators (Young *et al*., 1987).

Theobroma cacao is generally assumed to be an outbreeding species, because of its floral morphology and the occurrence of a self-incompatibility system (e.g. Knight and Rogers, 1955; Falque *et al*., 1995). However, while self-pollination of a single flower is basically impossible in *Theobroma* due to the flower morphology (Lieberei and Reisdorff, 2007), it has been suggested that geitonogamy might be more common in the wild than assumed (Lanaud *et al*., 1987). Chumacero de Schawe, *et al*. (2013) found selfing in wild and cultivated cacao trees. According to Winder and Silva (1972), the natural pollination in terms of fertilization rate of cacao reaches just 4% at the peak of the flowering season. Ceratopogonid midges are considered to be the principal and most effective pollinators of cacao, at least in cultivars (e.g. Billes, 1941; Posnette, 1950; Brew and Borman, 1993). Soria (1976) found a positive correlation between midge population levels and the intensity of flowering, fruit set, and yield. However, the quantity of flowers that become a ripe fruit in cacao is known to be lower than the pollination rate, because a large number of young fruits wilt (Wellensiek, 1932). Presumably for this reason, Young (1983) found no correlation between pollinator abundance and fruit set. Lately, Groenevald *et al*. (2010) obtained experimental evidence for stronger yield limitation by pollination than by plant resources. They also found an increase of pod abortion with pollination intensity. Saunders and

Bowman (1956) and Young (1983) pointed out that natural pollination seems to be a limiting factor in the production of cacao due to scarcity of *Forcipomyia* midges.

In view of the numerous uncertainties surrounding the pollination ecology of *T. cacao*, in our study we set out to document flower visitors in wild and cultivated plants of *T. cacao* in the northeast lowlands of Bolivia where presumed wild forms of cacao occur patchily in the natural forest. In addition, farmers cultivate commercial hybrids of both the *Forastero* and *Criollo* cultivars. The objectives of our study were: 1) to identify floral visitors and 2) to compare species composition and relative abundance of Diptera and Hymenoptera in wild and cultivated cacao flowers. The hypothesis of the study was that the pollinator assemblages differ between wild and cultivated populations of *T. cacao*, both with respect to taxonomic composition and abundance of floral visitors and that many flower visitor species, in addition to ceratopogonid midges, function as potential pollinators.

MATERIAL AND METHODS

Study species

The flowers of *Theobroma cacao* L. are pentamerous, white with red nectar guides, with 5 separate or fused petals ca. 10 mm long, consist of a proximal petal hood or pouch, each of which encloses an anther, have five rigid staminodes, an outer whorl of the androecium, surround the pistil like a fence and grow in clusters on the tree trunks. The fruits are ellipsoid to elongated, segmented (10 segments), 15-19 cm in length and greenish, orange or red when ripe, and are dispersed by gravity and mammals. Anthesis occurs at dawn and anther dehiscence takes place throughout the morning hours (Young *et al.*, 1987). Each flower produces about 14.000 pollen grains (Massaux *et al.,* 1976). Flowering phenology of *Theobroma cacao* is seasonal, varying between genotypes and in response to local climatic conditions, but usually being highest at the onset of the rainy season (Paulin *et al*., 1983). In Bolivia, the main flowering season is in September-October for wild trees and October-November for the cultivated ones, although some flowering occurs throughout the year among the cultivated trees (C. Chumacero, unpubl. data). The flowers are receptive for one day; stigma and style receptivity is high at morning and early afternoon (Young *et al*. 1987). Unpollinated flowers drop after about two days. *Theobroma cacao* is pollinated by insects and has no wind pollination (Harland, 1925). Ceratopogonid midges are considered to be the principal and most effective pollinators of cacao, at least in cultivars (e.g., Billes, 1941; Posnette, 1950; Brew and Borman, 1993). Flowers pollinated with sparse pollen grains fall without setting (Lachenaud, 1995). Less than 5% of the flowers set fruit in cacao fields (Entwistle, 1972).

Study sites and sampling

We studied flowers of cultivated and wild *Theobroma cacao* trees on the lands of the native Tacana community Napashi (67°52’17.4’’ S -14°5’20.6’’ W) in the northeast lowlands of Bolivia (Chumacero de Schawe, *et al.*, 2013). For our study, we selected 59 trees (26 from wild and 33 cultivated trees) from four cultivated plantations and from two patches of wild cacao trees. We sampled 2237 flowers (1077 from wild and 1160 cultivated trees) during 13 days between 14.10. and 16.11.2008, using “Insect Tangle Trap Glue” to catch pollinators of cacao. The marking time of the flowers was around 07:30 am, they were checked 24 hours later. We sampled a mean of 7 trees and 125 flowers per day, the number of flowers marked depended on the availability, as wild cacao trees produce much fewer flowers than the cultivars. Flowers with arthropods (N = 448) were stored in ethanol (70%) and the arthropods were rinsed with a solvent (Terpentin) to remove the glue. The insects were observed under the stereomicroscope to detect the presence of pollen, before and after the cleaning. All the specimens were determined to insect order level, Diptera and Hymenoptera were determined to morphospecies level, and Ceratopogonidae specimens were determined to species level. The Ceratopogonidae specimens were prepared in Canada balsam for determination. The samples were stored in the "Ceratopogonidae of the Neotropics Collection" at Universidad Nacional de la Plata in Argentina. The other Diptera specimens were stored in the "Colección Boliviana de Fauna", Mayor de San Andrés University, Bolivia. All the other insect samples are currently at the Agroecology Department of Georg August University of Göttingen, Germany.

Data analysis

We assessed the relative abundance of the floral visitors trapped on cacao flowers per order. Dominance was calculated as the relative abundance of the most abundant insect orders. In particular, species richness and relative abundance of Diptera and Hymenoptera were calculated.

We performed a G test to compare species richness and relative abundance of Diptera and Hymenoptera between wild and cultivated cacao. The index of Sorensen was calculated to compare the similarity in species composition of Diptera and Hymenoptera between both wild and cultivated cacao plants. Rank-abundance curves of Hymenoptera and Diptera species were plotted to compare the species richness in wild and cultivated cacao. We assessed chi square test to compare the proportion of species composition between wild and cultivated cacao.

RESULTS

The overall mean success capture of insects on cacao flowers was 21% with a mean of 0.3 captured insects per flower on both wild and cultivated cacao. A total of 631 insects were collected belonging to eight orders, 331 insects were found on wild and 300 on cultivated cacao trees. The order Hymenoptera was the most abundant group found on wild (n = 143) and cultivated (n = 118) cacao flowers. Diptera was the second group in abundance on wild (n = 79) and on cultivated cacao (n = 45) (Fig. 3.1).

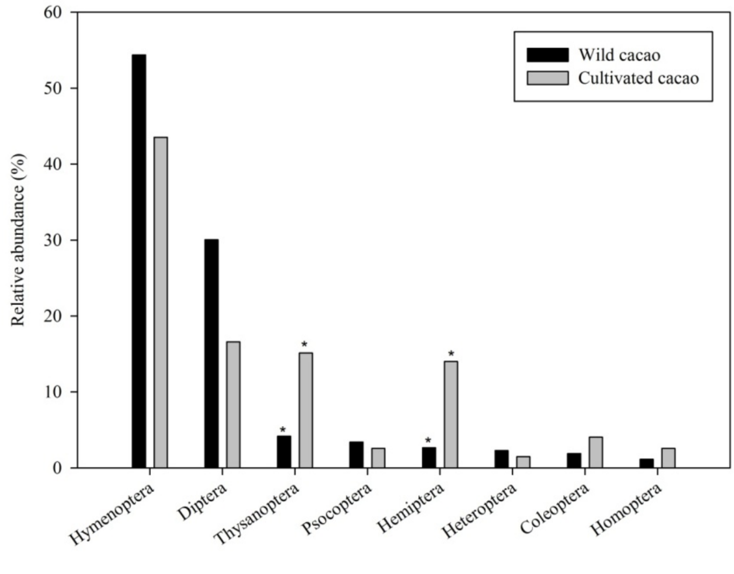

Fig. 3.1 Relative abundance of the insect visiting orders in wild and cultivated *Theobroma cacao*. $^*P < 0.05$.

We found 59 species of Hymenoptera belonging to 11 families and 49 species of Diptera belonging to 15 families. The abundance of Hymenoptera did not differ significantly between wild trees (54% of visitors) and cultivars (43%) (G-test; df =1; P = 0.31; Fig. 3.1).

Species richness of Hymenoptera was similar on cultivated (29 species) and on wild cacao trees (30). The most abundant family of Hymenoptera on wild cacao was Eulophidae (59%) and on cultivated cacao Plagygastridae (47%) (Table 3.1). Species composition of Hymenoptera between both cacao types was not very similar (Sorensen = 0.44). The proportions of Hymenoptera species do not differ significantly between wild and cultivated cacao (*Chi-square* test, df = 1, *P* = 0.085; Fig. 3.2A). The rank-abundance curve of Hymenoptera on wild and cultivated cacao, are quite similar, denoting similar species evenness in both cases (Fig. 3.2A). We found the presence of pollen only on a single specimen of Encyrtidae.

Table 3.1 Hymenoptera families trapped on flowers of wild and cultivated *Theobroma cacao*.

Family	**Wild** *n* (%)	**Cultivated** *n* (%)
Agaonidae	0	1(1)
Braconidae	2(1)	0
Ceraphronidae	3(2)	2(2)
Encyrtidae	5(4)	3(3)
Eulophidae	83(59)**	30(26)**
Eupelmidae	1(1)	0
Formicidae	3(2)*	16(14)*
Mymaridae	0	1(1)
Platygastridae	40(29)	54(47)
Scelionidae	0	3(3)
Trichogrammatidae	0	2(2)
Indet	3(2)	2(2)
Total	140	114

* P < 0.05; ** P < 0.001

The abundance of Diptera was not significantly different between wild (30% of visitors) and cultivated cacao (17% of visitors, *G*-test; df = 1; *P* = 0.078). Species richness of Diptera did not differ between wild (30 species) and cultivated cacao (19) (*G*-test; df = 1; *P* = 0.152) (Fig. 3.1). The most abundant families of Diptera were Chloropidae, Phoridae and Ceratopogonidae on both wild and cultivated cacao (Table 3.2). However, the species composition of Diptera and wild and cultivated cacao differed considerably (Sorensen = 0.33). The proportion of Diptera species differed significantly between wild and cultivated cacao, accordingly the rank-abundance curves have different shape (*Chi-square* test, df =1, *P* = 0.002; Fig. 3.2B). Additionally, the slope on wild cacao is stepper than the slope on cultivated cacao, denoting lower species evenness.

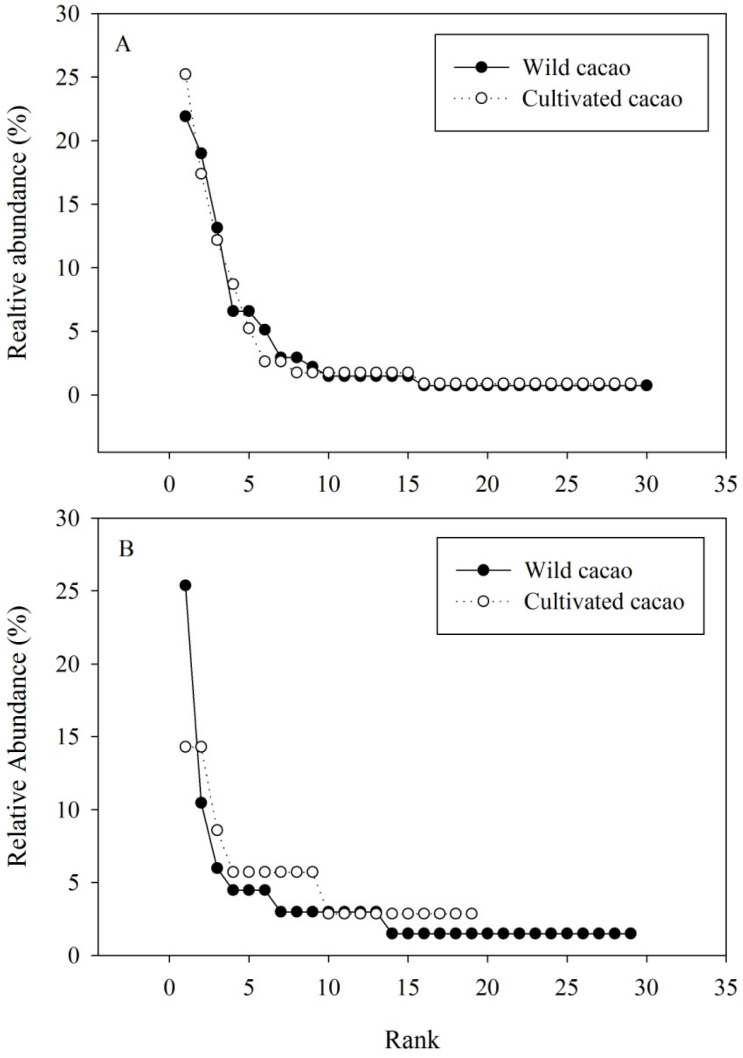

Fig. 3.2 Rank-abundance curves of A) Hymenoptera and B) Diptera in wild and cultivated *Theobroma cacao*.

Ceratopogonidae represented 12% of the Diptera found on wild cacao flowers, including five species, whereas this family represented 13% of the Dipterian flies trapped on cultivated cacao flowers, including three species (Table 3.3). Only *Forcipomyia (Thyridomyia) nana* (Macfie) was found on both wild and cultivated cacao flowers. No pollen grains were found on Ceratopogonidae midge's bodies. Except for a single specimen of *Culicoides* sp. 2, all the midges were females. Only one specimen of Cecidomyidae was found on wild cacao flowers. The abundance of Sciaridae in both wild and cultivated cacao was similarly low (*G*-test; df = 1; *P* = 0.615).

Table 3.2 Diptera families trapped on flowers of wild and cultivated *Theobroma cacao*.

Family	**Wild** *n* (%)	**Cultivated** *n* (%)
Asteiidae	0	1(2)
Camillidae	1(1)	0
Carnidae	2(3)	0
Cecidomyidae	1(1)	0
Ceratopogonidae	9(12)	6(13)
Chloropidae	21(27)	11(27)
Drosophilidae	1(1)	0
Empididae	5(6)	0
Phoridae	15(19)	12(29)
Muscidae	2(3)	0
Mycetophylidae	3(4)	0
Psychodidae	0	1(2)
Psilidae	1(1)	2(5)
Sciaridae	1(1)	3(7)
Simulidae	7(9)*	0*
Tachinidae	2(3)	1(2)
Indet.	7(9)	4(9)
Total	78	41

* $P < 0.05$

The relative abundance of the orders Thysanoptera (4% and 15% on wild and cultivated cacao, respectively; *G*-test; df = 1; *P* = 0.019) and Hemiptera (2% and 20% on wild and cultivated cacao, respectively; *G*-test; df = 1; *P* = 0.0012) were significantly higher on flowers of cultivated cacao. Hemiptera was represented mainly by aphids and Thysanoptera by thrips. Only three spiders were captured on cacao flowers.

Table 3.3 Ceratopogonidae species trapped on wild and cultivated *Theobroma cacao* flowers.

Specie	Wild	Cultivated
Culicoides fluviatilis (Lutz)	2	-
Culicoides sp. 1	-	1
Culicoides sp. 2	1	-
Forcipomyia (Euprojoannisia) sp. 2	1	-
Forcipomyia (Lepidohelea) sp. 1 (*annulatipes* group)	-	1
Forcipomyia (Thyridomyia) jipajapae Wirth	1	-
Forcipomyia (Thyridomyia) nana (Macfie)	4	2
Total	9	4

DISCUSSION

The relatively low capture rate of insects on cacao flowers (21%) evidenced the low abundance of floral visitors. Both wild cacao and cultivated cacao showed a low rate of capture. Wild cacao trees had fewer flowers (37 versus 56 per tree; C. Chumacero, unpublished data).

We found pollen only on a single specimen of the family Encyrtidae. Partly, this may be due to the collecting process that involves trapping with glue followed by rinsing and cleaning, even though this method has previously been used to identify cacao pollinators (Groenevald, 2008). One possibility would be that most insects were caught during their first visit to a flower, so that no pollen load was present from a previous visit. On the other hand, previous studies have also reported low pollen load on flower visitors (Winder, 1977) as well as pollen limitation in fruit production on cacao trees (Bos *et al.* 2007). In any case, regardless of the reason for the extremely low number of insects with pollen, we were unfortunately unable to directly identify the actual pollinators and were only able to compare the visitors.

The most abundant and diverse insect order on both wild and cultivated cacao trees was Hymenoptera. The taxonomic composition of the hymenopteran assemblages was largely similar between wild and cultivated cacao, although the family Eulophidae was the most abundant family on wild cacao whereas on cultivated cacao it was the Plagygastridae. Most species of Eulophidae are parasitoids whose larvae feed on insects or spiders while some species are known to parasitize Thysanoptera (Hanson and Gauld, 1995). Species of Plagygastridae are exclusively parasitoids. Adult parasitoids need sugar for their survival. In this sense, they might pollinate flowers of cacao while they suck nectar. Diptera was the second most commonly collected insect order on cacao flowers in our study but only a low proportion corresponded to midges of the family Ceratopogonidae that is commonly widely considered to be the main pollinators of cacao (e.g., Billes, 1941; Posnette, 1950; Brew and Borman, 1993). In our pollinator survey, we trapped 13 ceratopogonid specimens belonging to seven species. This low number of Ceratopogonidae found on cacao flowers supporting previous observations by Entwistle (1972) and Groenevald (2008) who caught only 28 Ceratopogonids during extensive sampling in cultivated cacao plantations in Indonesia. Among the seven species recorded by us, only *Forcipomyia* (*Thyridomyia*) *nana* was present in both wild and cultivated cacao flowers. A larger sample size would be necessary to test if the species composition of ceratopogonid midges differs between wild and cultivated cacao. According to our observations, species composition of ceratopogonid midge assemblages in Bolivia differs from those in other studies. We thus did not find any of the ceratopogonid species reported by Winder (1997) in Brazil or by Sánchez *et al*. (2001) in Venezuela. In contrast to other studies (e.g., Winder, 1977), we only trapped one Cecidomyiidae midge on wild cacao.

Among the remaining Diptera, Chloropidae and Phoridae flies were abundant. Chloropidae are mainly phytophagous, while Phoridae has scavenger, herbivore, parasitoids and predator species (Brown, 1992). The species of these families might carry cacao pollen when they feed on flower tissue. Furthermore, Phoridae are known to pollinate species of the genus *Herrania* (Young, 1984), a genus that is closely related to *Theobroma* (Cuatrecasas, 1964). Accordingly, Young (1984) suggested that Phoridae might play a role in cacao pollination.

The rank-abundance curves of Hymenoptera on wild and cultivated cacao showed a similar shape denoting a similar proportion of species, where the high-ranking species were more abundant than the low ranking species. The same morphospecies of Eulophidae was the most abundant species on both wild cacao and cultivated cacao. In contrast, the proportion of Diptera differed between both wild and cultivated cacao. Accordingly the slopes of the rank-abundance curve were different; it was steeper on wild cacao denoting lower species evenness than on cultivated cacao.

The relatively high abundance of Hemiptera found on cacao flowers evidenced pest infested cacao cultivars, while aphids in wild cacao trees were very rare. It has been discussed if aphids are cacao pollinators but Billes (1941) found evidenced that they are not involved in cacao pollination. In our study aphids were found on the outer sites of the flowers mainly on the pedicels thus we think that aphids do not pollinate cacao flowers. Thrips on the other hand, could contribute to pollinate cacao flowers; they were found on the petal pouch and inside the flowers. Some authors considered already thrips as pollinator agents (Pound, 1910; Billes, 1941; Entwistle, 1972, Soria *et al.*, 1980) although they transport very few pollen grains.

The assemblage of floral visitors of a certain plant consists of a variety of insects that vary in time and space (Herrera, 1988; Cane and Payne, 1993; Kandori, 2002). We thus cannot exclude the possibility that the low abundances of ceratopogonid midges on cacao flowers was a temporal phenomenon or that their role as visitors was at least partly compensated by visits of other groups of small Diptera and microhymenoptera. However, our study was conducted at the peak flowering period of both the wild and cultivated cacao trees in the study region (C. Chumacero *et al*., unpublished data) so that the pollinator assemblages observed by us presumably correspond to those responsible for a large part of the pollination events in cacao in the study region.

Although we were unable to identify the actual pollinators of the cacao plants in our study systems, we documented a wide range of flower visitors as well as considerable differences between the visitors of wild and cultivated cacao. Ceratopogonid midges were so infrequent that it is unlikely that they were the main pollinators in the study system. However, among the other insect groups, there are a number of potential additional pollinators, including small Diptera and Hymenoptera that may carry pollen when they feed on flowers tissue or suck nectar. The size of the visiting insect is known to be important for efficient pollination of cacao flowers and Soria *et al*. (1980) considered medium sized midges (0.9-0.3 mm) as the most effective pollinators. Most of the Diptera and Hymenoptera found by us fall in this size range and may thus fit the bill as pollinators of cacao in the study region.

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ACKNOWLEDGEMENTS

The authors thank the German Science Foundation (HE 3041/17-1) for funding this project. We also thank the German Academic Exchange Service (DAAD) for the scholarship granted to CC. For assistance in the field, we thank R. Hurtado, C. Campos, and the local assistants J. Navi, C. Ibaguari, E. Amutari and W. Amutari. For insect's identification, we thank G. Spinelli and P. Marino (Universidad Nacional de la Plata, Argentina), I. Vollhardt (University of Göttingen, Germany) and M. Limachi (Universidad Mayor de San Andrés, Bolivia). We also thank the National Herbarium of Bolivia (LPB) for logistic support, and the Ministerio de Desarrollo Rural y Medio Ambiente – Dirección General de Biodiversidad y Áreas Protegidas in Bolivia for research permits.

CHAPTER IV

SMALL-SCALE SPATIAL GENETIC STRUCTURE OF THREE WILD SPECIES OF *THEOBROMA* (MALVACEAE)

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(Manuscript to be submitted to *Heredity*)

ABSTRACT

Spatial genetic structure SGS allowed estimate gene dispersal distance within populations. This has important implications for the management and conservation of forest genetic resources. We studied three species of the genus *Theobroma*, wild *T. cacao*, *T. speciosum* and *T. subincanum* in two areas in Bolivia. These species are cauliflorus understory trees, have small flowers, and have heavy fruits with fleshy seeds. We assessed genetic diversity and small-scale spatial genetic structure (SGS) and we inferred gene dispersal distances from the patterns of SGS. *T. speciosum* and *T. subincanum* showed relatively high genetic diversity, while *T. cacao* showed low genetic diversity. We found a significant spatial genetic structure overall individuals of the three *Theobroma* species, but *T. subincanum* showed the strongest SGS. The extent of SGS also varies among species. *T. subincanum* had the highest *Sp* value and *T. speciosum* showed the largest gene dispersal distance. Gene dispersal distances were intermediate for the three *Theobroma* species. Particularly in cacao, where both pollen dispersal and gene dispersal distances were assessed, the results suggest that seed dispersal is limited. The small-scale spatial genetic structure found in *Theobroma*, pointed out the importance of the protection of seed dispersal animals to maintain the genetic resources of this tropical tree genus.

Key words: seed dispersal, pollen dispersal, cacao, Bolivia

INTRODUCTION

Small-scale spatial genetic structure (SGS) is a result of different processes, most importantly as a consequence of limited gene dispersal that causes local pedigrees (Vekemans and Hardy, 2004). In turn, SGS allows to estimate gene dispersal distance within populations (Vekemans and Hardy, 2004; Jump *et al.*, 2012). As a descriptor of non-random spatial distribution of genetic variation, SGS has important implications for the management and conservation of forest genetic resources, e.g. for sampling designs (Epperson, 1992).

Previous studies found significant effects on SGS of breeding system, life form (Vekemans and Hardy, 2004), seed dispersal and population density (Hamrick and Trapnell, 2011; Zeng *et al.*, 2011). Thus – everything else being equal - more pronounced SGS is expected in selfing relative to outcrossing species, in small relative to large species, in weakly dispersed relative to well dispersed and in sparse relative to densely growing species. Most tropical species are outcrossed (Bawa, 1992; Chase *et al.*, 1996). Consequently, most studies on the genetic structure on tropical tree species have found high levels of intrapopulation genetic diversity and weak to moderate SGS (e.g., Hamrick and Loveless, 1989; Schierenbeck *et al.*, 1997; Konuma *et al.*, 2000; Lacerda *et al.*, 2001; Takeuchi *et al.*, 2004; Hardesty *et al.*, 2005, 2006). The dispersal of pollen in insect pollinated tree species is expected to be more limited than in species with bird pollen dispersal. Nevertheless, this was not reflected in spatial genetic structure (Degen *et al.*, 2001). In contrast, species with short seed dispersal displayed stronger genetic structure than plants with long distance seed dispersal (Degen *et al.*, 2001). Tree density may affect SGS through a number of processes. First, the density of trees and canopy gaps in the forest can influence the SGS because they affect light and microclimatic conditions determining the establishment of siblings (Born *et* *al*., 2008; Bizoux *et al*., 2009). In insect pollinated species the number or density of co-flowering species or individuals may cause competition for pollinators or facilitation with different effects on pollination (Mitchell *et al*., 2009). Competition for pollinator services among species probably affects gene flow within species causing changes in visit number and quality of pollinators (Mitchell *et al*., 2009). In populations at low density it has been suggested that the movement of pollinators among individuals could be less effective and as a consequence SGS tends to be stronger (Zeng *et al.*, 2012). Overall however, although there is a tendency of stronger SGS in species with animal vs. wind-dispersed pollen, and in species with gravity vs. animal-dispersed seeds, different types of pollen and seed dispersal did not consistently influence patterns of SGS (Vekemans and Hardy, 2004). Thus, more empirical studies may be needed to unravel more general patterns.

The genus *Theobroma* L. comprises about 20 species of understory trees native to the neotropics. Studies on the genetic diversity have mostly been restricted to non-natural populations of cultivated *Theobroma cacao* L. that has been widely distributed (e.g. Lanaud, 1987; Lerceteau *et al*., 1997). However, Silva *et al*. (2011) studied a wild population of cacao in Brazil. Cultivars showed high levels of genetic variability, although some *Criollo* cultivars had reduced diversity (Lercetau *et al*., 1997; Motamayor *et al*., 2002). However, genetic variation was lower in wild populations from southwestern Amazonia – the presumed center of diversity of *T. cacao* (Silva *et* al., 2011; Thomas *et al.* 2012) - than in planted populations in lower Amazonia (Sereno *et al*., 2006). This was interpreted as suggesting that wild populations of cacao have a higher degree of self-fertilization than cultivated ones. Nevertheless, Chumacero de Schawe, *et al*. (2013) found higher selfing in cultivated than in wild trees.

Here, we study three species of the genus *Theobroma*, wild *T. cacao*, *T. speciosum* Willd. ex Spreng. and *T. subincanum* Mart. These three species are cauliflorus understory trees, with numerous small flowers, and have heavy fruits with fleshy seeds. Small insects pollinate these species, although there is no published data on pollination of *T. subincanum*, but its floral morphology suggests that is presumably pollinated by insects. The pollen of wild cacao is dispersed long distances (Chumacero de Schawe, *et al*., 2013). The pods of the study species are dispersed by gravity and mammals, e.g. by squirrels (Emamdie and Warren, 1993) and presumably also by monkeys (Horacio Lorini, per.comm.).

The objectives of our study were: 1) to assess genetic diversity of populations of three common *Theobroma* species in Bolivia; 2) to compare the small-scale spatial genetic structure and; 3) to infer gene dispersal distances from the patterns of SGS.

MATERIAL AND METHODS

Study species

The genus *Theobroma* (Malvaceae, formerly Sterculiaceae) has 18-20 species distributed in the humid forests of Central and South America, many of which are widespread as a result of cultivation (Macbride, 1956). The presumed center of origin of the genus is in the upper waters of the Amazon tributaries Napo, Putumayo (Ica River in Brazil) and Caquetá (Japura River in Brazil) along the Andean base (Chessman, 1944). In Bolivian Amazonia six wild species of *Theobroma* are found: *T. cacao*, *T. speciosum*, *T. obovatum* Klotzch ex Bernoulli, *T. subincanum*, *T. bicolor* Bonpl. and, *T. microcarpum* Mart. (LPB, 2006; TROPICOS, 2006).

Our study encompasses three understory, shade tolerant, cauliflorous *Theobroma* species that are among the most common and widespread species of the genus. These species have hermaphroditice flowers, are mainly outcrossed, and are presumably pollinated by insects (Cope, 1958; Falque *et al*., 1995; Souza and Venturieri, 2010).

Theobroma cacao L. is widespread in humid lowland forests from southern Mexico to Bolivia and Brazil. Because of its long history of cultivation, the native range of *T. cacao* is uncertain, but is most probably located in southwestern Amazonia including Bolivia (Whitkus *et al.*, 1998; Motamayor *et al.*, 2002; Sereno *et al.*, 2006). It has been proposed that the "wild" populations of *T. cacao* in Bolivia are the result of previous cultivation (Tratado de Cooperación Amazónica, 1999). However, cultivated and wild cacao plants are genetically distinct (Chumacero de Schawe, *et al.*, 2013), supporting the notion that Bolivia harbours truly wild cacao populations. Wild cacao grows taller (up to 18 m) than cultivated cacao (to 6 m) (Lieberei and Reisdorff, 2007). Flowers are small (corolla diameter 14mm) and white with red nectar guides growing in clusters on the tree trunks (Cuatrecasas, 1964; Santos *et al.*, 2012) and in thin branches in the crown. The number of flowers of wild trees is low in wild cacao compared with cultivated varieties (Chumacero, C., unpublished data). Tiny midges pollinate wild cacao flowers (Ceratopogonidae, Diptera; Wellensiek, 1932; Soria *et al.*, 1980). The fruits are 12 cm long, smaller than the cultivated ones, globose to fusiform, with smaller seeds, more pulp, and thicker fruit shells. The seeds are 2.72 cm length, are dispersed by gravity, monkeys and sometimes by humans (Table 4.1). Squirrels eat ripe pods (Emamdie and Warren, 1993).

Table 4.1: Life history traits related to reproductive strategies of *Theobroma* spp.

Species	**Pollinators**	**Seed dispersers**	**Growth strategy**	**Seed size**	**Flower size**
T. speciosum	Drosophilidae	Mammals / Gravity	Shade tolerant	1.86 cm	14 mm
T. subincanum	Nocturnal insects	Mammals / Gravity	Shade tolerant	1.83	19 mm
T. cacao	Ceratopogonidae /Insects	Mammals (monkeys, squirrels) / Gravity	Shade tolerant	2.72	14 mm

Theobroma speciosum Willd. ex Spreng. is widespread in lowland Amazonia below 300 m in Ecuador, Peru, Brazil, and Bolivia. The trees reach 6-18 m in height, the flowers are small (14mm), dark red developing in clusters on the trunks (Cuatrecasas, 1964; Santos *et al.*, 2012). In Brazil, this species is pollinated by various species of drosophilid and phorid flies (Diptera; Azevedo-Rodriguez da Silva and Martins, 2006). The fruits are ellipsoid, segmented (7 segments), 10-12 cm in length and olive green, yellow when ripe (Cuatrecasas, 1964) with on average 8.1 seeds per pod (Santos *et al.*, 2012). Seeds are 1.8 cm in length (Santos *et al.*, 2012, Table 4.1). They are edible, and the local people prepare a sweet drink and chocolate from the seeds. There is some evidence of fruit consumption by monkeys (Leonardo Tube, per. comm.).

Theobroma subincanum Mart. is widespread in lowland Amazonia below 300 m in Colombia, Venezuela, the Guyanas, Surinam, Ecuador, Peru, Brazil, and Bolivia. The trees reach 6-12 m up to 20 m in height. The flowers are slightly larger (19mm) and dark red (Cuatrecasas, 1964; Santos *et al.*, 2012, Table 4.1) growing on canopy branches. In Bolivia the flowering occurs mainly at the end of the dry season and the beginning of the wet season (September-November). The pollinators of *T. subincanum* are unknown but are presumably small nocturnal insects (Rodrigues and Venturieri, 1997). The fruits are 7-12 cm long, ellipsoidal, brown, and are used to make sweet drinks from the pulp (Villacres, 1995). Seeds are 1.83 cm length (Santos *et al.*, 2012).

The species differ in flower color, sepals color, flower fragrance, style length, position of inflorescences, fruit size and possible pollinators (Santos *et al.*, 2012) (Table 4.1). In *T. speciosum* inflorescences are located only on the trunk, in *T. cacao* both on the trunk and on canopy branches and in *T. subincanum* exclusively on canopy branches. The flowers of *T. speciosum* are red and perpendicular to the trunk of the tree and exposed. In contrast the white to pink flowers of *T. cacao* are pendant. *Theobroma cacao* has longer staminodes than the other two species. Pods of *T. cacao* are larger than those of *T. speciosum* and *T. subincanum* (Santos *et al.*, 2012). According to Young *et al.* (1984) and Erickson *et al.* (1986) differences in floral fragrance are evident among species of *Theobroma*. *Theobroma speciosum* is pollinated by drosophilid and phorid flies (Azevedo-Rodriguez da Silva and Martins, 2006) whereas tiny midges pollinate *T. cacao* flowers, mainly ceratopogonids (Wellensiek, 1932; Soria *et al.*, 1980). *T. subincanum* is presumably pollinated by nocturnal insects (Rodrigues and Venturieri, 1997). Finally the three species differ in patterns of reproduction with wild *T. cacao* being partially self-compatible whereas *T. speciosum* is known to be outcrossing species (Souza and Venturieri, 2010). There is no published data on sexual reproduction of *T. subincanum* but is presumably outcrossed.

Study sites and sampling

The study was conducted in lowland Bolivia. The study sites are primary forest, although to a certain degree impacted by extraction of timber and non-timber products. *Theobroma speciosum* and *T. subincanum* were studied at the “Forest Reserve El Tigre” (65°43’8.7’’S – 10°58’38.8’’ W) close to Riberalta town in Beni (Fig. 4.1a). *Theobroma cacao* was studied on the lands of the native Tacana community Napashi (67°52’17.4’’ S -14°5’20.6’’ W) in the northeast lowlands of Bolivia (Fig. 4.1b) where presumed wild forms of cacao occur patchily in the natural forest. Leaf material was collected from all adult trees in an area of 2 x 0.5 km in Riberalta and 6 x 0.4km in Napashi resulting in a total of 112 samples of *Theobroma speciosum*, 73 samples of *T. subincanum*, and 155 samples of wild *T. cacao*. For the estimation of summary statistics of genetic variation groups of spatially close trees were formed: ten groups in *T. cacao*, four in *T. speciosum* and three groups in *T. subincanum*. Group size ranged between 4 and 41 (mean = 20). Tree density was estimated for each species in two plots of 1 ha.

Microsatellite analysis

We used the genotype data of wild *T. cacao* assessed by Chumacero de Schawe, *et al.*, (2013). DNA extraction of *T. speciosum* and *T. subincanum* was performed with a standard protocol adapted from Doyle & Doyle (1987) using 20mg silica-gel-dried leaf material and a modified extraction buffer [2% alkyltrimethylammoniumbromide (ATMAB), 0.1 M TRIS – HCl, 0.02 M disodium-EDTA (pH 8.0), 1.4 M NaCl, 1% polyvinylpyrrolidone]. The microsatellite loci established for *Theobroma cacao* by Lanaud *et al*. (1999) and Saunders *et al*. (2004) were optimized for these species, adding different volumes of $MgCl_2$ (MP Biomedicals, Solon, Ohio, USA) in order to improve the amplification of the alleles (Appendix 4.4). Samples were genotyped at five microsatellite loci in five simple reactions: mTcCir25, mTcCir11, mTcCir 12, mTcCir15 + mTcCir21 (annealing temperature 46°C).

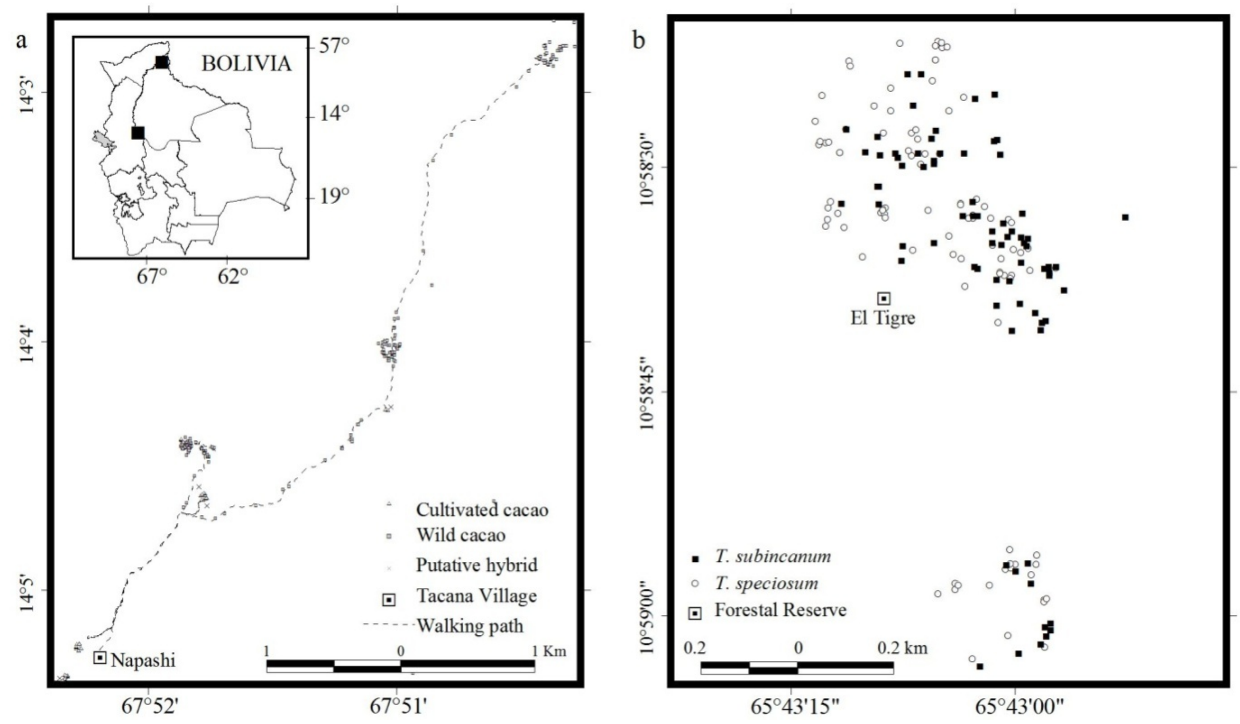

Fig.4.1 Location map of the study areas and the sampled trees. a) *Theobroma cacao*, b) *Theobroma speciosum* and *Theobroma subincanum*

PCR assays were set up in final volumes of 25 µl, containing 20 ng/µl of genomic DNA, 1 µl of primer (5 pmol/mL; Metabion International AG, Martinsried, Germany), 2.5 µL dNTPs (2 mM; Q-Biogene, Montreal, Quebec, Canada), 0.2 µl Taq DNA polymerase (5U / µl, MP Biomedicals), 2.5 µl incubation mix T. Pol with 1.5 mM $MgCl_2$ (MP Biomedicals) and 16.8 µl H_2Obidest. Note that the volume of H_2Obidest varied according to the volume of $MgCl_2$ added for each primer (Appendix 4.4). PCR was performed in a Mastercycler gradient or Mastercycler epgradient (Eppendorf, Hamburg, Germany) under the following temperature regime: 94°C for 4 min, 32 cycles with 30 s at 94°C, 60 s at 46°C, and 60 s at 72°C, and a final 3 min at 72°C. 1 µl PCR products (1:5 diluted) were used for separation on a MegaBace sequencer 1000 system (Amersham Bioscience, Uppsala, Sweden) with MegaBace-ET Rox 400 (Amersham Bioscience) as a size standard. The genotyping was performed with the MegaBace Fragment Profiler software 1.2 (Amersham Bioscience)

Data analysis

Genetic diversity was assessed as expected heterozygosity (H_E), number of alleles (A) and allelic richness (AR) ($n = 338$) in Fstat 2.9.3.2 (Goudet, 2001). We compared diversity levels among the three species by resampling 1000 times in Fstat. The Kruskal-Wallis test was performed to compare the genetic diversity (H_E, A and AR) between wild and cultivated cacao.

Small-scale spatial genetic structure (SGS) was assessed first by computing kinship coefficients (Loiselle *et al.*, 1995) between pairs of individuals within given distance classes using SPAGeDI V1.3d (Hardy and Vekemans, 2002). We defined 12 distance classes for all the species (25, 50, 75, 100, 125, 150, 175, 200, 250, 500, 1000 and 2500 m). Autocorrelograms were rated significant when at least one distance class had a significant kinship coefficient. Significance and 95% confidence intervals for $F_{(d)}$ were calculated based on 999 random permutations of individuals among geographical locations. Intensity of SGS was quantified by the *Sp* statistic, which represents the rate of decrease in pairwise kinship with distance (Vekemans and Hardy, 2004). *Sp* was calculated as $-b/(1-F_{(1)})$ where $F_{(1)}$ is the mean F_{ij} between individuals in the first distance class. Gene dispersal distance (σ) was estimated in SPAGeDI V1.3d (Hardy and Vekemans, 2002). The effective density for each species was calculated as one half and one-tenth of the census density (Vekemans and Hardy, 2004).

SGS was also assessed using spatial autocorrelation of the *r*–coefficient (Smouse and Peakall 1999) in GenAlEx 6.5b3 (Peakall and Smouse, 2006). We formally compared SGS among species with a heterogeneity test (Smouse *et al*., 2008). Test statistics ω and t^2 were obtained based on 999 permutations.

RESULTS

Genetic diversity

The gene diversity differed among the three species with *T. speciosum* showing the highest levels of gene diversity, allelic richness and total number alleles, *T. subincanum* intermediate values and *T. cacao* showing the lowest levels (Table 4.2).

Table 4.2: Genetic variation of *Theobroma* spp.

Species	*n*	*δ*	H_E	AR	A
T. speciosum	112	14.5	0.742*	8.49*	42
T. subincanum	73	9.5	0.672*	5.98*	30
T. cacao (wild)	155	1.3	0.424*	3.43*	17

N, number of samples*;* δ, density (tree/Ha); H_e, gene diversity*;* A_r*,* allelic richness*, A,* total number of alleles
**p* < 0.05, ***p* < 0.01.

Small-scale spatial genetic structure

Both, the analysis of kinship coefficients and the correlation coefficient ($\omega = 50.5$, $p = 0.001$) revealed a significant overall small-scale SGS in the three *Theobroma* species (Fig. 4.2). The autocorrelation coefficient *r*, declined with increasing distance for all species (Fig. 2b), with *T. subincanum* showing stronger SGS ($\omega = 101.7$, $p = 0.001$) than *T. cacao* (wild) ($\omega = 86.2$, $p = 0.001$), and *T. speciosum* ($\omega = 78.4$, $p = 0.001$). The extent of SGS also varied among species, *T. speciosum* SGS extent to 125 m, *T. subincanum* to 125 m (250) and wild cacao to 75 m (Fig 4.2a). At some distance classes the three species had significantly different SGS. In particular, *T. subincanum* had higher autocorrelation than *T. speciosum* at 50, 250, 1000 and 2500 m (Fig. 4.2b). As expected from the autocorrelograms, the *Sp* statistic, which represents the rate of decrease in pairwise kinship with the distance, was highest in *T. subincanum* (*Sp* = 0.048) whereas *T. speciosum* and *T. cacao* showed similarly lower values (*Sp* = 0.004; *Sp* = 0.007, respectively) (Table 4.3).

Indirect estimates of gene flow

The estimated gene dispersal distance was largest for *Theobroma speciosum* at 208 m and 310 m, considering 0.5 and 0.1 of effective densities, respectively. *Theobroma subincanum* and wild *T. cacao* showed a similar gene dispersal distances. For wild *T. cacao* it was 114 m considering 0.5 effective density; and for *T. subincanum* it was 59 and 121 m considering 0.5 and 0.1 effective densities respectively (Table 4.3).

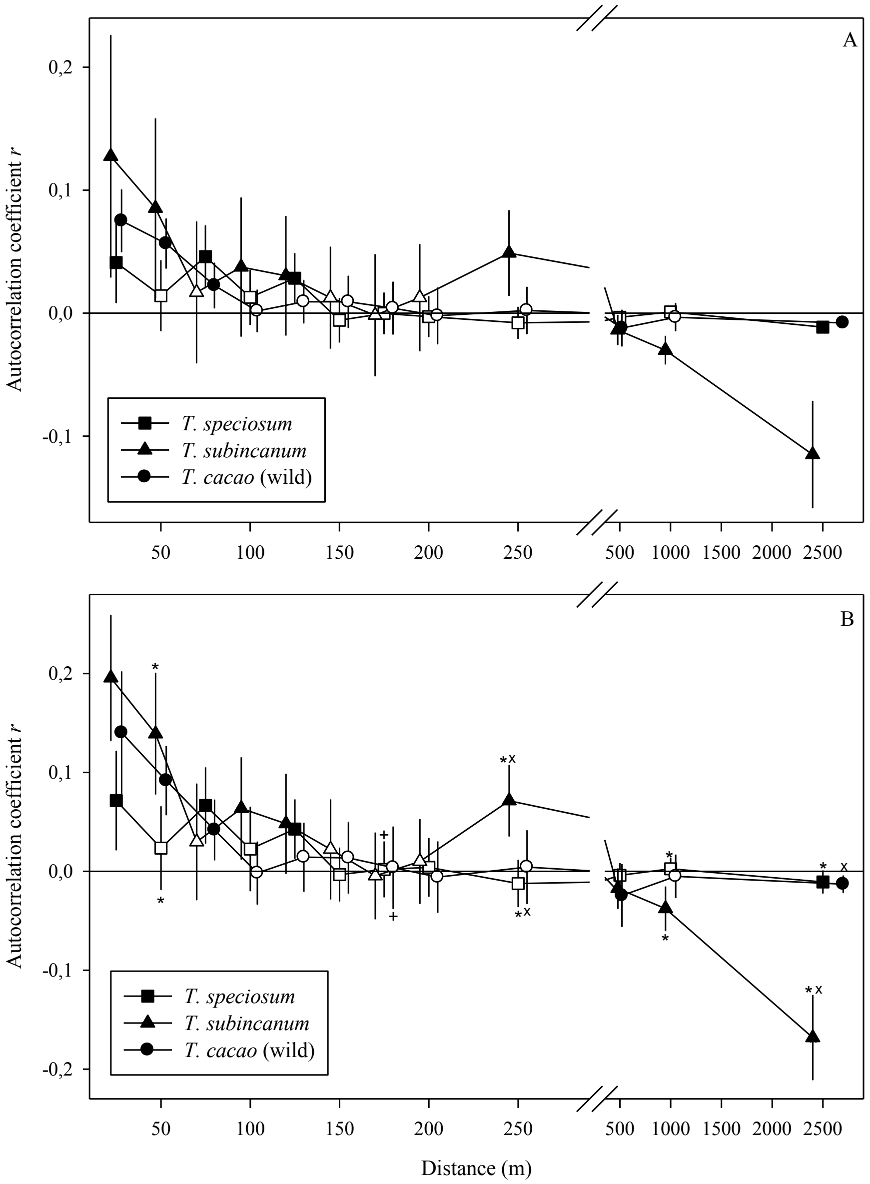

Fig. 4.2. Genetic similarity among *Theobroma speciosum*, *T. subincanum* and *T. cacao* (wild), expressed as autocorrelation coefficient *r*. Note that symbols are displaced within each distance class for better visibility. Symbols are filled when individuals are significantly more similar or dissimilar at a distance class than expected by chance. Differences between pairs of species as determined by heterogeneity tests ($p < 0.01$) are indicated by symbols * + x near the respective data points.

Table 4.3 Spatial genetic structure and gene dispersal distance of *Theobroma* spp.

Specie	ω	σ (m)		$F_{(1)}$	$b_{\log}$	*Sp*
		0.5D	0.1D			
T. speciosum	78.4*	208	310	0.0411	-0.0039 **	0.0041
T. subincanum	101.7*	59	121	0.1275	-0.0417**	0.0478
T. cacao (wild)	86.2*	101	-	0.0699	-0.0064**	0.0069

ω, autocorrelation coefficient; $F_{(1)}$, kinship coefficient in the first distance class; $b_{\log}$, regression slope of spatial genetic autocorrelation; σ, *mean* dispersal distance; *Sp,* statistic.
**p* < 0.05, ***p* < 0.01

DISCUSSION

Genetic diversity

Genetic diversity differed among the three species with *T. speciosum* and *T. subincanum* showing higher levels of diversity than *T. cacao*. Low levels of genetic diversity had also been found in wild cacao populations compared to cultivated cacao (Sereno *et al.*, 2006; Chumacero de Schawe *et al.*, 2013). This may be due to several causes. First, it has been suggest that wild populations of cacao have a higher degree of self-fertilization than cultivated cacao (N´Goran *et al.*, 2000; Sereno *et al.*, 2006; but see Chumacero de Schawe *et al.* (2013). In turn, the high level of genetic diversity in *T. speciosum* and *T. subincanum* suggest that they have outcrossing breeding systems. Second, among the three species, only *T. cacao* has been widely used and cultivated by local people.

Small-scale spatial genetic structure

The three species of *Theobroma* displayed low, but significant small-scale spatial genetic structure. However, spatial genetic structure was more pronounced in *T. subincanum* than in the other species as evidenced by the low levels of the *Sp* statistic in *T. speciosum*

(0.004) and wild *T. cacao* (0.007) compared to *T. subincanum* (*Sp* = 0.048). Recently, an intermediate *Sp* value was observed in a dense population of *T. cacao* (*Sp* = 0.0209; Silva *et al.*, 2011), however based on a smaller sampling area, indicating that SGS may be both context dependent, e.g. with respect to disturbance level, and parameter estimates may depend on sampling scale. Compared to other species studied by Vekemans and Hardy (2004), *T. speciosum* had similar *Sp* value to *Dycorinia guianensis* (*Sp* = 0.005), an animal pollinated tree with mixed animal / gravity dispersed seeds. Whereas *T. subincanum* had a similar *Sp* value to *Vouacapoua americana*, a tropical tree species (*Sp*=0.039) with a predominantly outcrossing breeding system, respectively. Wild *T. cacao* had a similar *Sp* value to temperate herbs, *Lesquerella fendleri* (Brassicaceae) (*Sp*=0.007) and *Chamaecrista fasciculata* (Fabaceae) (*Sp*=0.007), with self-incompatibility and mixed mating system, respectively.

For *T. subincanum* we observed strongly negative autocorrelation coefficients in the last distance classes. It might be argued that this could correspond to the comparison between two genetically differentiated patches (Fig. 4.1). However, results were identical when only trees in the larger patch were analyzed (data not shown), probably because a ravine acts as geographic barrier, separating the two clusters.

Gene dispersal distance

The gene dispersal distance estimated for *T. speciosum* is comparable with *Aucoumea klaineana*, a predominantly outcrossing, insect pollinated tree species that has wind dispersal (210-570 m, Born *et al.*, 2008). The values found for *T. subincanum* are comparable with the predominantly outcrossing *Fagus sylvatica*, which is pollinated by wind and the seeds are dispersed by animals (65 – 145 m, Oddou-Muratorio *et al.*, 2010). *T. cacao* (wild) has similar gene dispersal distance than *Prunus avium*, a self- incompatible, and insect pollinated and animal dispersion tree species (106 m, Schueler *et al.*, 2006).

The largest gene dispersal distance was found in *T. speciosum* (208 - 310 m), suggesting a more efficient seed and pollen dispersal. We found some solitary trees of *T. speciosum* in open areas; we assumed that such trees connect patches and trees located far away from each other. We observed solitary trees in an open area produced much more pods than trees inside the forest. Perhaps the flowers in such trees are more visible to attract pollinators. The attraction of pollinators is important for the success of sexual reproduction in insect pollinated plants. In many plant species, the flower color, flower size, flowering intensity show differences in pollinator attraction, although in *Theobroma* apparently the differences in flower colors do not affect pollination between species (Santos *et al.*, 2012). Young *et al*. (1984) and Erickson *et al*. (1986) evidence differences in floral fragrance among species of *Theobroma*. Nevertheless, microclimatic conditions seem to influence more pollination success than morphological differences.

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ACKNOWLEDGEMENTS

The authors thank the German Science Foundation (HE 3041/17-1) for funding this project. We also thank the German Academic Exchange Service (DAAD) for the scholarship granted to CC. For assistance in the field, we thank R. Hurtado, C. Campos, and the local assistant L. Tube. Special thanks to Birgit Müller and S. Träger for the assistance in the lab. We also thank the National Herbarium of Bolivia (LPB) for logistic support, the Ministerio de Desarrollo Rural y Medio Ambiente – Dirección General de Biodiversidad y Áreas Protegidas in Bolivia, and the University of Riberalta for research permits.

APPENDIX 4.1. Genotype of *Theobroma speciosum*

ID	**Group**	**MTcCir25**		**MTcCir11**		**MTcCir12**		**MTcCir15**		**MTcCir21**		**Coordx**	**Coordy**
347	1	124	128	0	0	220	220	230	230	172	182	1457.22784	3112.31472
309	1	130	132	364	364	216	228	230	230	182	182	1513.74182	3112.31472
341	1	128	132	360	360	242	242	230	230	170	182	1335.27662	3188.93347
310	1	124	132	364	364	222	222	230	230	182	182	1522.66508	3118.44422
343	1	130	130	360	362	228	228	230	230	170	182	1391.7906	3250.22846
342	1	128	130	360	364	216	216	230	230	182	182	1388.81618	3259.42271
345	1	128	132	360	364	212	212	230	230	170	182	1370.96966	3072.47297
344	1	132	132	364	364	222	222	230	230	174	182	1439.38132	3167.48022
313	1	130	132	360	364	212	212	230	230	174	182	1513.74182	3066.34347
308	1	124	132	364	366	242	242	230	230	170	182	1555.3837	3219.58097
316	1	124	132	360	360	250	250	230	230	172	182	1540.5116	3069.40822
318	1	130	130	360	366	222	222	230	230	182	182	1561.33254	3262.48746
348	1	130	148	360	364	214	214	230	230	182	182	1472.09994	3158.28597
315	1	130	132	360	360	242	252	232	254	170	180	1531.58834	3047.95498
306	1	124	130	364	364	212	212	232	254	178	178	1472.09994	3204.25722
319	1	130	132	360	364	212	212	232	254	170	176	1588.10113	3158.29087
320	1	124	132	360	364	242	256	232	254	180	180	1507.79298	3075.53772
346	1	128	132	364	364	216	216	232	254	170	180	1370.96966	3072.47297
307	1	128	130	360	360	222	222	232	254	170	182	1489.94646	3296.19971
314	1	124	130	360	364	216	220	232	254	166	176	1531.58834	3047.95498
311	1	128	132	360	364	222	222	232	254	172	172	1525.64188	3100.05817
317	1	130	132	360	364	212	212	232	254	170	172	1561.33254	3262.48746
312	1	128	130	360	364	220	220	232	254	170	178	1570.25104	3069.40822
723	1	130	132	360	364	220	244	230	230	172	182	1328.85187	3089.2678
732	1	128	132	360	364	212	212	230	230	180	180	1616.89471	3185.25577
768	1	130	132	362	364	242	242	240	240	170	170	1342.77216	3092.57773
772	1	128	148	362	364	242	262	230	230	184	184	1575.13385	3287.86359
805	1	128	130	362	364	216	216	230	234	178	184	1566.56752	3300
823	1	132	132	364	364	212	222	230	230	172	184	1349.19691	3093.68104
826	1	124	130	360	360	214	220	230	230	170	184	1584.77097	3288.9669
850	1	124	130	364	364	220	220	230	230	168	184	1561.21356	3291.17352
857	1	130	132	360	366	222	262	230	230	170	182	1330.99346	3094.78435
864	1	124	130	360	364	212	222	230	230	170	184	1383.46222	3120.16048
900	1	124	130	360	364	0	0	230	230	178	184	1322.42713	3136.71013
938	1	124	132	364	364	256	256	230	230	170	184	1572.99227	3297.79338
327	2	124	128	360	362	228	228	230	234	172	182	1713.02796	2928.42973
330	2	128	130	360	364	260	260	230	230	172	182	1730.87448	2867.13474
323	2	130	130	360	366	222	222	230	232	182	182	1635.69304	2943.75348
339	2	130	130	360	362	222	222	230	230	182	182	1597.02558	2864.06999
336	2	130	130	360	364	222	222	230	230	170	182	1713.02796	2821.16349
340	2	130	132	360	366	226	226	230	230	182	182	1588.10232	2900.84699
322	2	124	130	360	360	242	242	230	230	172	182	1626.76978	2937.62398
328	2	124	130	366	366	222	222	230	230	182	182	1674.3605	2882.45849
337	2	128	154	360	366	248	248	230	230	166	182	1716.00238	2873.26424
321	2	128	130	364	366	0	0	230	230	172	182	1635.69304	2937.62398

ID	**Group**	**MTcCir25**		**MTcCir11**		**MTcCir12**		**MTcCir15**		**MTcCir21**		**Coordx**	**Coordy**
333	2	130	132	360	364	220	220	230	234	172	182	1689.2326	2827.29299
324	2	130	132	360	364	222	242	232	254	170	176	1659.4884	2962.14198
332	2	130	130	360	360	256	256	232	254	172	172	1698.15586	2821.16349
335	2	128	130	364	366	220	220	232	254	166	180	1692.20702	2854.87574
338	2	132	132	360	364	212	212	232	254	170	180	1611.89768	2854.87574
325	2	128	132	360	366	222	222	232	254	170	176	1671.38608	2937.62398
329	2	130	142	360	364	220	220	232	254	180	184	1745.74658	2876.32899
334	2	124	130	352	358	222	222	232	254	180	182	1689.2326	2824.22824
326	2	130	130	364	364	222	222	232	254	166	166	1707.07912	2934.55923
331	2	0	0	0	0	220	252	0	0	0	0	1748.721	2830.35774
202	2	132	156	348	360	216	222	234	252	170	170	1415.58596	2858.67603
746	2	130	132	360	360	220	264	230	264	164	172	1710.05354	2814.54363
754	2	124	130	362	364	220	256	230	230	176	184	1367.40036	2948.04413
771	2	138	138	348	368	260	260	230	258	170	184	1516.24033	2871.91575
773	2	124	132	360	366	216	216	230	230	172	184	1642.59369	2976.73019
781	2	130	132	362	366	218	218	230	230	168	184	1451.99286	2949.14744
789	2	128	132	360	360	218	218	230	262	170	184	1611.54075	2967.90371
803	2	132	132	360	364	220	242	230	230	184	184	1546.22249	2954.66399
809	2	124	148	362	364	212	216	230	230	178	184	1685.42534	2724.07222
816	2	124	130	362	364	212	212	230	248	172	182	1800	2835.50652
831	2	142	174	350	372	258	258	232	256	184	184	1620.10708	2797.99398
853	2	130	132	360	364	212	212	230	230	178	184	1379.17906	2918.25476
859	2	128	130	360	366	212	216	230	230	172	184	1352.40928	2971.21364
868	2	130	130	360	360	228	262	230	230	178	184	1345.98453	2934.80441
881	2	128	130	360	360	212	212	230	230	170	184	1460.55919	2959.07723
883	2	130	132	364	366	240	242	230	230	178	184	1459.4884	2946.94082
905	2	0	0	0	0	0	0	0	0	170	184	1611.54075	2965.69709
920	2	130	130	360	364	216	216	230	230	184	184	1456.27603	2956.87061
922	2	132	132	360	360	222	222	230	230	178	184	1460.55919	2939.21765
937	2	128	130	362	362	212	212	230	230	184	184	1456.27603	2953.56068
953	2	128	128	360	364	212	212	230	230	172	184	1342.77216	2921.56469
726	3	124	128	364	364	212	212	230	230	182	182	1719.69066	2227.58275
727	3	124	130	364	364	260	260	230	230	178	178	1777.51338	2154.76429
749	3	124	130	360	360	216	216	230	230	172	184	1565.49673	2168.00401
757	3	128	132	360	360	256	256	230	230	170	184	1634.02736	2034.50351
767	3	124	132	362	364	212	212	230	230	182	182	1777.51338	2151.45436
776	3	130	132	362	364	216	216	230	250	184	184	1782.86734	2158.07422
787	3	128	128	360	364	244	244	230	240	172	182	1604.04521	2187.86359
798	3	124	132	364	364	212	212	230	230	172	184	1761.45152	2227.58275
814	3	130	130	360	360	242	256	230	230	172	184	1705.77037	2081.94584
835	3	130	130	360	364	214	214	230	230	170	184	1708.98275	2258.47543
838	3	130	132	364	366	220	220	230	244	170	182	1778.58418	2057.67302
858	3	124	130	364	366	212	212	230	240	172	184	1600.83284	2176.83049
901	3	128	130	360	360	220	260	230	230	170	184	1762.52231	2246.33902
909	3	132	132	360	364	220	220	230	276	184	184	1600.83284	2188.9669
927	3	130	132	364	364	218	222	230	240	170	184	1751.8144	2206.61986

ID	**Group**	**MTcCir25**		**MTcCir11**		**MTcCir12**		**MTcCir15**		**MTcCir21**		**Coordx**	**Coordy**
928	3	128	130	364	364	212	220	230	230	170	184	1606.18679	2184.55366
932	3	132	132	360	360	212	212	230	230	172	184	1711.12433	2219.85958
940	3	128	132	360	364	212	218	230	276	184	184	1700.41642	2217.65296
962	3	124	132	364	366	222	250	230	230	184	184	1710.05354	2229.78937
963	3	132	132	360	360	220	240	248	248	184	184	1668.29268	2184.55366
187	4	132	148	360	360	232	254	232	254	170	178	98.51279	565.997994
188	4	174	174	346	346	220	220	232	254	170	180	1275.31231	905.817452
189*	4	146	146	348	368	258	258	232	254	178	180	607.138608	781.14343
190	4	148	174	346	352	252	258	232	254	166	166	1060.08328	923.470411
197	4	130	154	362	362	216	216	234	254	182	182	476.502082	810.932798
199	4	124	130	360	364	256	274	234	254	170	170	193.813206	759.077232
200	4	128	130	364	364	214	214	234	234	170	170	1302.08209	915.747242
201	4	132	156	348	360	216	222	232	254	170	170	1299.94051	919.057172
203	4	124	132	360	364	212	248	234	252	170	170	78.1677573	478.83651
204	4	124	132	360	360	246	270	234	254	170	170	0	0
205	4	128	132	360	364	222	242	234	234	170	170	8.56632957	185.356068
206	4	124	132	360	364	214	214	234	254	170	170	1299.94051	913.540622
735	4	130	132	360	364	212	218	230	230	182	182	194.182629	759.239418
736	4	128	128	360	364	220	220	230	230	172	182	194.182629	759.239418
737	4	130	150	362	362	242	242	230	230	170	184	194.182629	759.239418
738	4	130	130	364	366	216	222	230	268	172	184	194.182629	759.239418

APPENDIX 4.2. Genotype of *Theobroma subincanum*

ID	Group	MTcCir25		MTcCir11		MTcCir12		MTcCir15		MTcCir21		Coordx	Coordy
379	1	148	148	344	372	258	262	232	242	182	182	1327.1862	2703.59969
352	1	138	142	368	372	258	262	256	256	182	182	1496.72814	2685.21119
357	1	128	130	344	352	260	262	232	234	182	182	1633.55146	2651.49894
353	1	154	154	344	368	258	258	232	256	182	182	1469.95836	2654.56369
383	1	0	0	344	368	252	258	232	254	182	182	1449.13742	2816.99543
382	1	136	164	344	346	252	260	234	242	182	182	1461.0351	2752.63568
356	1	136	136	344	368	258	260	242	254	182	182	1627.60262	2682.14644
349	1	136	136	344	344	262	262	232	254	182	182	1514.57466	2654.56369
384	1	0	0	344	368	258	260	234	242	182	182	1475.9072	2816.99543
354	1	136	136	344	368	252	258	232	254	182	182	1502.67698	2633.11044
350	1	136	160	344	354	252	260	232	234	182	182	1481.85604	2626.98094
381	1	148	148	344	346	258	262	242	254	182	182	1389.64902	2688.27594
358	1	124	130	368	368	260	260	232	236	182	182	1502.67698	2639.23994
380	1	136	136	344	372	252	258	232	254	182	182	1365.85366	2657.62844
355	1	0	0	344	344	252	252	232	256	182	182	1621.65378	2679.08169
975	1	156	174	368	368	258	262	232	254	138	138	1562.28435	2654.56369
976	1	138	148	346	368	260	262	232	244	182	182	1623.31945	2774.82447
978	1	136	174	346	346	252	262	234	254	182	182	1429.50625	2645.73721
981	1	136	136	372	372	260	260	232	258	182	182	1425.22308	2654.56369
984	1	140	174	346	372	252	260	230	230	138	138	1583.70018	2767.1013
986	1	136	174	348	372	252	252	234	256	138	138	1395.24093	2651.25376
1000	1	174	174	346	348	258	262	234	256	138	138	1438.07258	2629.18756
351	1	138	138	344	346	258	260	232	254	166	166	1505.6514	2700.53494
364	2	146	146	346	368	252	262	232	234	182	182	1392.62344	2550.3622
366	2	140	140	344	358	244	258	232	254	182	182	1389.64902	2587.1392
359	2	138	138	346	368	260	262	234	256	182	182	1588.93516	2525.8442
374	2	148	148	368	368	258	258	232	242	182	182	1687.09102	2464.5492
371	2	148	148	344	372	258	258	232	234	182	182	1618.67936	2470.6787
360	2	138	148	346	368	260	262	234	256	182	182	1580.0119	2525.8442
365	2	148	148	368	368	252	258	232	256	182	182	1392.62344	2587.1392
373	2	136	148	344	372	252	252	232	256	182	182	1681.14218	2470.6787
377	2	138	138	344	368	252	260	232	254	182	182	1582.98632	2421.64271
376	2	0	0	368	372	252	260	232	254	182	182	1588.93516	2418.57796
363	2	136	148	344	368	252	260	232	254	182	182	1440.21416	2464.5492
368	2	124	130	372	372	260	260	232	254	182	182	1657.34682	2495.1967
362	2	0	0	346	372	252	252	232	254	182	182	1437.23974	2433.90171
372	2	148	148	368	372	260	260	254	254	168	182	1636.52588	2467.61395
375	2	138	138	346	368	258	260	234	254	168	182	1675.19334	2430.83696
367	2	154	154	344	368	258	260	234	254	182	182	1648.42356	2482.9377
361	2	0	0	344	344	258	262	232	232	166	182	1502.67698	2470.6787
370	2	138	138	344	368	252	258	254	254	182	182	1618.67936	2495.1967
964	2	140	140	346	346	252	258	234	256	138	138	1717.54908	2307.02106
965	2	136	136	346	348	252	262	256	256	138	138	1730.39857	2421.7653
966	2	160	174	346	368	258	262	256	256	138	138	1761.45152	2374.32297

ID	**Group**	**MTcCir25**		**MTcCir11**		**MTcCir12**		**MTcCir15**		**MTcCir21**		**Coordx**	**Coordy**
970	2	174	174	346	372	252	262	230	230	138	138	1318.14396	2550.85256
971	2	174	174	346	352	252	262	230	256	138	138	1673.64664	2346.74022
973	2	136	140	346	346	262	262	232	258	138	138	1677.9298	2530.99298
977	2	136	140	346	368	258	258	244	256	138	138	1723.97383	2311.4343
980	2	148	174	346	368	260	262	234	258	182	182	1675.78822	2482.44734
982	2	136	174	346	346	252	260	232	232	182	182	1721.83224	2417.35206
983	2	174	174	346	368	260	262	244	244	182	182	1714.3367	2292.67803
985	2	136	174	346	372	252	258	256	256	138	138	1703.62879	2326.88064
990	2	140	140	346	348	252	262	234	256	138	138	1731.46936	2404.11234
992	2	148	174	346	346	252	258	230	234	0	0	1731.46936	2409.62889
994	2	156	174	346	348	252	258	232	234	182	182	1688.63772	2480.24072
995	2	136	174	346	348	258	258	244	256	138	138	1652.23081	2393.07924
996	2	174	174	358	368	252	258	242	256	138	138	1626.53183	2342.32698
998	2	174	174	346	368	258	262	230	256	138	138	1745.38965	2421.7653
999	2	136	136	346	346	252	262	230	256	138	138	1626.53183	2395.28586
1002	2	136	174	372	372	258	260	234	244	138	138	1579.41701	2555.2658
378	2	138	138	368	372	258	260	232	256	176	180	1559.19096	2525.8442
369	2	138	138	346	368	258	258	232	254	182	182	1639.5003	2510.52045
968	3	174	174	368	372	252	252	234	234	138	138	1734.68174	1677.03109
969	3	136	148	346	368	252	252	234	256	138	138	1733.61095	1690.27081
974	3	128	128	368	368	258	258	230	256	138	138	1695.06246	1773.01906
979	3	136	148	346	368	252	252	230	256	138	138	1645.80607	1810.53159
987	3	174	174	368	368	252	252	234	258	138	138	1593.3373	1603.10933
988	3	148	174	346	346	252	252	258	258	138	138	1726.11541	1663.79137
989	3	136	148	346	368	252	262	234	244	138	138	1670.43427	1629.58877
991	3	136	174	368	368	252	258	242	256	0	0	1688.63772	1813.84152
993	3	136	174	346	368	258	260	244	256	138	138	1664.00952	1797.29188
997	3	148	174	346	368	258	262	234	244	138	138	1714.3367	1648.34504
1001	3	174	174	346	368	258	258	234	242	138	138	1722.90303	1683.65095

APPENDIX 4.3 Genotype of *Theobroma cacao* (wild)

ID	Group	MTcCir25		MTcCir11		MTcCir12		MTcCir15		MTcCir21		Coordx	Coordy
434	1	134	140	292	292	220	246	236	236	170	172	3411.18	7810.18
435	1	136	140	292	292	220	236	236	236	170	170	3471.60	7751.32
436	1	134	140	292	310	212	220	236	236	170	170	3450.02	7762.72
437	1	134	140	292	310	220	236	236	236	170	170	3439.23	7794.99
438	1	134	140	292	292	220	246	236	236	170	170	3511.52	7726.64
439	1	140	140	292	292	212	220	236	236	170	170	3515.84	8300.00
440	1	134	134	290	292	210	220	236	236	170	172	3529.87	7810.18
442	1	140	140	290	310	220	220	236	236	170	172	3600.00	7984.84
443	1	134	140	290	290	236	246	236	236	170	172	3454.34	7872.83
444	1	140	140	292	292	220	236	236	236	170	172	3460.81	7891.82
445	1	140	140	292	292	220	220	236	236	170	170	3454.34	7950.67
446	1	140	140	292	292	220	220	236	236	170	170	3453.26	7986.74
447	1	134	134	292	292	236	236	236	236	170	172	2514.54	7935.48
448	1	134	140	290	292	220	246	236	236	170	172	3442.47	7981.05
449	1	134	136	292	292	220	220	236	236	170	170	3485.63	8024.71
450	1	134	140	290	292	220	220	236	236	170	170	3405.78	7832.96
451	1	134	140	292	292	236	246	236	236	170	172	3406.86	7817.77
452	1	134	136	292	292	220	236	236	236	170	172	3415.49	7851.95
453	1	148	148	292	292	220	246	236	256	170	170	3248.25	7464.65
403	2	134	140	292	292	212	246	236	236	148	170	2322.48	4273.22
404	2	134	134	292	310	212	212	236	236	170	170	2291.19	4250.43
405	2	134	134	292	292	220	236	236	236	170	170	2259.90	4233.35
406	2	136	140	292	292	220	236	236	252	170	170	2335.43	4043.49
407	2	134	136	290	292	220	236	236	236	170	170	2301.98	4075.77
408	2	136	140	292	292	220	236	236	252	170	170	2298.75	4229.55
409	2	134	136	292	292	220	246	236	236	170	170	2328.96	4018.81
410	2	134	140	0	0	236	236	236	236	170	170	2333.27	4018.81
411	2	134	140	292	310	220	246	236	236	170	170	2365.64	3948.57
412	2	134	136	290	292	212	212	252	260	160	170	2377.51	4009.32
413	2	134	136	290	292	236	246	252	252	170	170	2369.96	4028.31
414	2	136	140	292	292	220	220	236	252	170	170	2375.35	4087.16
415	2	134	140	292	292	212	220	236	252	160	170	2375.35	4130.83
416	2	134	140	290	310	246	246	236	260	170	170	2396.93	4168.80
417	2	134	140	290	292	220	220	252	260	170	170	2408.80	4202.97
418	2	136	140	292	292	204	236	236	236	170	172	2408.80	4210.56
419	2	136	140	292	292	236	246	236	236	170	170	2412.04	4225.75
66	4	134	140	292	292	212	236	236	236	170	170	3550.84	7917.66
67	4	134	140	292	310	204	212	234	236	170	170	3552.71	7945.18
68	4	134	134	292	292	236	246	236	236	170	170	3488.07	7771.74
69	4	134	176	190	190	220	236	236	236	170	172	3600.00	7976.91
70	4	134	134	292	292	220	236	236	236	170	170	3565.98	7945.18
71	4	134	140	292	292	220	220	236	236	170	170	3491.08	7811.36
72	4	134	140	292	292	220	236	236	236	170	170	3506.15	7861.38
73	4	134	134	292	292	236	246	236	236	170	172	3556.19	7976.85

ID	Group	MTcCir25		MTcCir11		MTcCir12		MTcCir15		MTcCir21		Coordx	Coordy
74	4	134	134	292	292	220	220	236	236	170	170	3458.09	7781.37
75	4	134	140	290	292	236	246	236	236	170	170	3541.34	7919.51
76	4	134	140	292	310	220	236	236	236	172	172	3581.92	7984.23
77	4	134	136	292	292	212	236	236	236	170	172	3596.08	8277.05
78	4	134	140	292	310	212	246	236	236	170	170	3451.30	7791.95
79	4	140	140	292	292	212	212	236	236	170	170	3566.55	7924.09
80	4	132	140	292	292	212	236	236	236	170	170	3501.83	7818.41
81	4	140	140	292	310	220	236	236	236	170	170	3472.80	7741.25
82	4	134	140	292	292	212	236	236	236	170	170	3510.69	7843.72
458	6	134	154	292	292	220	220	236	236	170	170	1044.97	2805.65
459	6	134	134	292	292	220	220	236	236	170	170	1042.81	2748.70
460	6	134	134	292	292	212	220	236	236	170	170	1041.73	2813.25
461	6	140	150	292	292	246	246	236	252	170	170	1035.25	2809.45
462	6	134	150	292	292	220	220	236	236	160	170	1035.25	2820.84
463	6	140	140	292	292	220	220	236	256	170	170	1035.25	2841.72
464	6	140	140	290	292	220	220	236	236	170	170	1020.15	2885.39
465	6	134	140	292	292	212	220	236	236	170	170	1010.44	2875.90
466	6	136	140	290	290	220	220	236	236	170	170	1010.44	2864.51
467	6	140	148	292	292	212	246	236	254	170	170	1012.60	2858.81
468	6	134	140	292	292	0	0	236	236	160	160	1014.75	2856.91
471	6	134	136	292	292	212	236	236	236	170	170	995.33	2921.46
472	6	136	136	292	292	220	246	236	236	160	170	994.25	2913.87
454	7	134	134	292	292	246	246	236	236	170	172	863.70	2177.24
455	7	134	148	292	292	212	212	236	236	170	170	884.20	2228.50
456	7	134	140	292	292	220	246	236	236	170	170	3085.32	2255.08
457	7	140	140	292	292	220	236	236	250	170	172	941.38	2564.54
385	8	134	140	292	292	212	220	236	236	160	170	995.33	2048.14
387	8	134	140	292	292	212	220	236	236	170	170	1156.10	2093.70
388	8	134	136	292	292	212	220	236	236	170	170	1381.61	2200.02
389	8	134	140	292	292	220	246	236	256	170	170	1617.91	2433.54
390	8	140	140	292	292	212	220	236	236	170	170	1572.59	2401.26
391	8	134	140	292	292	212	236	236	236	170	170	1876.86	2767.68
392	8	140	140	292	292	220	246	236	236	170	170	1995.55	2919.56
393	8	134	134	292	292	212	212	236	236	170	170	2002.02	2915.77
394	8	140	140	292	292	204	204	236	236	170	170	2062.45	3023.98
395	8	134	148	292	292	220	246	236	252	170	170	2072.16	3003.10
396	8	140	140	292	292	220	220	236	236	170	170	2068.92	3033.47
397	8	134	134	292	292	212	246	236	236	170	170	2062.45	3082.84
398	8	134	134	292	292	212	236	236	236	170	170	2109.92	3217.63
399	8	140	140	290	292	220	220	236	250	170	172	2114.24	3213.84
400	8	140	140	292	310	220	246	236	236	160	170	2136.90	3272.69
31	9	140	140	292	292	220	220	234	236	170	170	12.19	48.39
46	11	134	140	292	292	212	236	236	260	170	170	2295.36	4112.27
722	11	134	140	290	292	204	220	236	236	172	172	2381.85	4210.88
47	11	136	140	292	292	220	220	236	236	170	170	2367.70	4218.79
48	11	134	140	292	310	212	236	236	236	170	170	2289.69	4124.84

ID	**Group**	**MTcCir25**		**MTcCir11**		**MTcCir12**		**MTcCir15**		**MTcCir21**		**Coordx**	**Coordy**
49	11	134	136	292	292	220	236	236	236	170	170	2351.71	4120.74
50	11	140	150	290	292	212	246	236	236	170	170	2285.33	4130.17
51	11	134	134	292	292	212	220	236	236	170	170	2330.77	4051.42
52	11	136	140	290	292	220	236	252	252	170	170	2294.75	4159.09
53	11	134	134	292	294	220	236	236	236	170	170	2361.18	4112.48
54	11	134	140	292	310	220	246	236	236	170	170	2309.79	4224.76
55	11	134	140	290	310	220	220	236	236	170	170	2314.14	4077.72
56	11	136	140	292	292	212	220	236	236	170	170	2353.40	4128.82
57	11	134	140	292	292	220	220	254	260	170	170	2353.88	4072.34
58	11	134	140	292	310	220	246	236	236	170	172	2355.38	4064.35
59	11	134	140	290	292	212	220	236	236	170	170	2339.40	4121.56
60	11	134	140	292	294	220	220	236	236	170	170	2355.48	4064.29
61	11	134	140	292	292	212	220	236	236	170	170	2299.39	4121.58
62	11	134	140	292	292	212	220	236	236	170	170	2361.09	4127.05
63	11	132	138	290	292	212	220	236	236	170	170	2331.40	4208.99
64	11	134	140	292	292	220	236	236	236	170	170	2287.46	4203.67
65	11	134	140	292	292	212	220	236	252	170	170	2337.83	4213.06
19	12	134	140	290	292	212	236	236	236	170	170	885.74	3012.96
1	13	134	140	292	292	246	246	236	236	160	170	909.01	2989.81
2	13	140	148	290	292	246	246	236	236	160	170	903.62	2978.42
3	13	148	148	290	292	220	246	236	236	170	170	887.19	2953.18
4	13	140	148	292	292	220	246	236	236	160	170	892.83	2938.55
5	13	134	134	292	292	246	246	236	236	172	172	890.53	2960.02
6	13	134	140	292	302	204	246	236	236	160	172	887.61	2985.73
7	13	126	130	292	292	204	246	236	236	172	172	888.34	2995.82
8	13	132	140	292	302	212	246	236	236	170	172	899.62	3005.66
9	13	134	140	292	292	246	246	236	236	170	170	909.17	2998.35
10	13	140	148	290	292	220	246	236	236	160	170	907.23	2984.51
420	15	134	136	292	292	212	220	236	236	170	170	2374.27	4296.00
421	15	134	134	292	292	220	236	236	236	170	170	2376.43	4341.56
422	15	134	140	292	292	236	246	236	236	170	172	2373.20	4436.49
423	15	134	140	292	292	212	220	236	236	170	170	2368.88	4438.39
424	15	140	140	292	292	212	220	236	236	170	170	2366.72	4430.79
425	15	134	140	292	292	220	220	236	236	170	170	2372.12	4544.71
426	15	134	134	292	292	220	236	236	236	170	170	2400.17	4556.10
427	15	134	134	292	310	220	220	236	236	170	172	2386.14	4632.04
428	15	134	140	292	292	212	246	236	236	170	170	2640.78	4968.08
429	15	140	140	292	292	212	220	236	236	170	170	2578.20	5404.74
430	15	136	140	292	292	236	246	236	236	170	170	2503.75	90.75
431	15	134	134	292	292	236	236	236	236	160	170	2650.49	6536.26
432	15	134	134	290	290	220	246	236	236	170	170	2779.97	6864.71
433	15	134	134	292	292	246	246	236	236	170	170	3333.49	7667.79
475	16	134	140	292	292	204	236	236	236	170	172	917.65	2986.01
476	16	134	140	292	292	236	236	236	236	170	172	912.25	3006.90
477	16	140	148	292	292	246	246	236	236	170	170	911.17	3035.37
478	16	140	140	290	292	220	220	236	236	170	170	878.80	3056.26

ID	Group	MTcCir25		MTcCir11		MTcCir12		MTcCir15		MTcCir21		Coordx	Coordy
509	16	148	148	292	292	204	220	234	236	170	170	852.91	2925.26
510	16	134	134	292	292	220	220	236	236	170	170	886.36	2828.43
511	16	134	140	292	292	220	220	236	236	170	170	1081.65	2925.26
512	16	134	136	292	292	212	236	236	252	170	170	1068.70	2929.06
513	16	134	154	292	292	204	236	236	236	170	170	859.38	2904.37
514	16	134	140	290	292	220	220	236	236	170	170	904.70	2887.29
515	16	140	140	290	292	204	220	236	236	170	170	898.22	2870.20
516	16	134	136	290	292	220	246	236	236	170	172	897.15	2908.17
517	16	134	134	292	292	220	236	236	236	170	170	903.62	2891.09
518	16	140	140	290	292	220	246	236	236	170	170	905.78	2927.16
519	16	134	140	290	292	212	220	236	236	170	170	915.49	2936.65
520	16	140	140	292	292	218	246	236	236	170	170	910.09	2923.36
521	16	134	136	290	292	204	212	236	236	170	170	907.93	2927.16
522	16	136	136	292	292	220	246	236	236	170	170	903.62	2923.36
523	16	140	140	292	292	220	246	236	236	170	170	915.49	2948.04
524	16	134	140	292	292	220	246	236	236	170	170	921.96	2944.24
525	16	134	134	290	292	204	246	236	236	170	170	910.09	2961.33
526	16	148	148	290	292	246	246	236	236	160	170	915.49	2974.62
527	16	140	140	292	292	236	246	236	236	160	172	951.09	2989.81

Appendix 4.4 Modified protocols for microsatellite analysis of *Theobroma speciosum* and *T. subincanum*

MTcCir25 protocol for *Theobroma speciosum*

	Multiplex Cir25	
	µl 1x	**µl x106**
Buffer	2,5	265
Forward	0,5	53
Rev	0,5	53
dNTP	2,5	265
Magnesium	**1,2**	127,2
Taq	0,2	21,2
Bidest water	16,6	1759,6
Mastermix	**24**	2544
DNA	1	

MTcCir25 protocol for *Theobroma subincanum*

	Multiplex Cir25	
	µl 1x	**µl x106**
Buffer	2,5	265
Forward	0,5	53
Rev	0,5	53
dNTP	2,5	265
Magnesium	**1,8**	190,8
Taq	0,2	21,2
Bidest water	16	1696
Mastermix	**24**	**2544**
DNA	1	

MTcCir11_12 protocol for *Theobroma speciosum*

	Multiplex Cir11/Cir12	
	µl 1x	**µl x110**
Buffer	2,5	265
Forward	0,5/0,8	53 / 84,8
Rev	0,5/0,8	53 / 84,8
dNTP	2,5	265
Magnesium	**3,2**	339,2
Taq	0,2	21,2
Bidest water	13	1378
Mastermix	**24**	**2544**
DNA	1	

MTcCir11_12 protocol for *T. subincanum*

	Multiplex Cir11/Cir12	
	µl 1x	**µl x110**
Buffer	2,5	265
Forward	0,5/0,8	53 / 84,8
Rev	0,5/0,8	53 / 84,8
dNTP	2,5	265
Magnesium	**2,0**	212
Taq	0,2	21,2
Bidest water	14,2	1505,2
Mastermix	**24**	**2544**
DNA	1	

MTcCir15_21 protocol for *Theobroma speciosum*

	Multiplex Cir15/Cir21	
	µl 1x	**µl x110**
Buffer	2,5	265
Forward	0,5/0,8	53 / 84,8
Rev	0,5/0,8	53 / 84,8
dNTP	2,5	265
Magnesium	**3,0**	318
Taq	0,2	21,2
Bidest water	13,2	1399,2
Mastermix	**24**	**2544**
DNA	1	

MTcCir15_21 protocol for *T. subincanum*

	Multiplex Cir15/Cir21	
	µl 1x	**µl x110**
Buffer	2,5	265
Forward	0,5/0,8	53 / 84,8
Rev	0,5/0,8	53 / 84,8
dNTP	2,5	265
Magnesium	**0,4**	42,4
Taq	0,2	21,2
Bidest water	15,8	1674,8
Mastermix	**24**	**2544**
DNA	1	

CHAPTER V

SYNTHESIS

This study shows that wild and cultivated cacao populations in northwestern Bolivia are genetically distinct, with cultivated trees showing higher genetic diversity. In contrast to prior studies self-pollination occurs at meaningful rates in *Theobroma cacao* especially among the cultivated trees. However, cross-pollination is prevalent and mainly takes place over distances of up to a few hundred meters. Accordingly, pollination mostly occurs within the spatially segregated cultivated and wild populations. However, long pollen dispersal distances were also observed. Gene flow between wild and cultivated *T. cacao* populations takes place. Further, a wide range of flower visitors were documented as well as considerable differences between the visitors of wild and cultivated *T. cacao*. The findings suggest that midges alone were probably too rare to act as main or even sole pollinators of *T. cacao* in our study region. Potential additional pollinators would be small Diptera and Hymenoptera. In respect to the wild relatives of *T. cacao*, *T. speciosum* and *T. subincanum* they showed relatively high gene diversity. I assessed SGS of these three species and the results showed that *Theobroma* species displayed low but significant small-scale spatial genetic structure. *T. speciosum* showed the largest pollen dispersal distance, whereas *T. subincanum* was the more structured species and had the lowest gene dispersal distance.

GENERAL DISCUSSION

This study clearly distinguished between cultivated and "wild" cacao plants in the study site in northwestern Bolivia, showing that the wild plants are genetically distinct from the currently cultivated ones which confirm the hypothesis addressed. While this is not conclusive evidence, it nevertheless suggests that the “wild” cacao populations of lowland Bolivia may correspond to truly wild forms or at least to ancient cultivars that have become naturalized a long time ago. This lends support to the assumption that Bolivia is part of the natural range of *Theobroma cacao* (Soria, 1969; Whitkus *et al*., 1998; Arguello *et al*., 2000; Motamayor *et al*., 2003; Sereno *et al*., 2006). Furthermore, we only found few individuals corresponding to the cultivated genotypes in the forest, which shows that currently, seed dispersal of cultivated forms into the forest (either by humans or animals) and successful establishment is infrequent.

Genetic diversity differed among the three *Theobroma* species, with *T. speciosum* and *T. subincanum* showing relatively high levels of genetic diversity while *T. cacao* showed low genetic diversity independently from the number of primer used to assess genetic diversity. Normally is expected that wild populations have higher genetic diversity, but my data revealed lower levels of genetic diversity in wild cacao populations compared to cultivated cacao. Probably, in the study area cultivated cacao has a higher genetic diversity because the cultivated forms belong to both major cultivars (*Forastero* and *Criollo*) and presumably come from a variety of origins. Furthermore, genetic variation of the Bolivian wild populations studied was also lower than values reported for wild cacao populations in Brazil (Sereno *et al*., 2006; Silva *et al*., 2010). This may be due to several causes. First, it has been suggested that wild populations of cacao have a higher degree of self-fertilization than cultivated cacao (N´Goran *et al*., 2000; Sereno *et al*., 2006) but I found higher self-fertilization in cultivars, despite the close proximity of cultivated plants, which should actually increase cross-pollination. The higher degree of self-pollination in cultivated cacao might be the result of human selection toward high crop production in cultivated plants. In turn, the high level of diversity in *T. speciosum* and *T. subincanum* suggest that they have outcrossing breeding systems. Second, among the three species, only *T. cacao* has been widely used and cultivated by local people.

Although self- pollination occur, outcrossing clearly predominates in *T. cacao* thus most pollination events documented in this study took place over distances of less than a few hundred meters, as is typical for many tropical species (e.g., Cloutier *et al*., 2007; Carneiro *et al*., 2009). However, the mean pollen dispersal distance found in *T. cacao* (867 m) was higher than that reported for other insect-pollinated tropical understory tree species. Pollen dispersal distances differed between wild and cultivated cacao, with longer pollen dispersal distances observed in wild cacao confirming the hypothesis of this study. Furthermore, I documented pollen dispersal distances over 2.5 km in single individuals in both wild and cultivated cacao. Such pollen dispersal distances are unusual for an insect-pollinated tropical understory tree species. In canopy trees, pollen dispersal distances tend to be larger, as exemplified by distances of 5.8-14.2 km in seven species of the genus *Ficus*, which are pollinated by small wasps (Nason *et al*., 1998). The large pollen dispersal distances in *T. cacao* are surprising considering the minute sizes of the pollinating ceratopogonid midges (0.5-2 mm). Although dispersal over long distances is poorly understood in insects, individuals of small species of Diptera, Homoptera, and Hymenoptera are known to be wind dispersed (Nason *et al*., 1998). However, such wind dispersal is unlikely in the interior of the forest understory and suggests either the insects disperse actively or that they emerge over the forest canopy to be transported in the aerial plankton, as also assumed for fig pollinating wasps (Nason *et al*., 1998).

As relatively little is known about the pollination ecology of *T. cacao* and because its assume that wild and cacao pollinators are the same, I tested the hypothesis that pollinator assemblages differ between wild and cultivated *T. cacao* populations, both with respect to taxonomic composition and abundance of floral visitors. I documented a wide range of flower visitors as well as considerable differences between the visitors of wild and cultivated cacao. Species composition of Hymenoptera between both cacao types was not very similar. However, species composition of Diptera differed significantly between wild and cultivated cacao. Further, the orders Thysanoptera and Hemiptera were more abundant on cultivated cacao. The most abundant and diverse insect order visiting flowers of both cacao types was Hymenoptera. Most of the hymenopteran visitors on cacao flowers probably corresponded to parasitoid species, whose adults need sugar for their survival. In this sense, they might pollinate flowers while they suck nectar. Diptera was the second most commonly collected insect order on cacao flowers, but only a low proportion corresponded to midges of the family Ceratopogonidae that is commonly widely considered to be the main pollinators of cacao (e.g., Billes, 1941; Posnette, 1950; Brew and Borman, 1993). In our pollinator survey, we trapped 13 ceratopogonid specimens belonging to seven species. This low number of Ceratopogonidae found on cacao flowers agrees with previous observations by Entwistle (1972) and Groenevald (2008) who caught only 28 ceratopogonids during extensive sampling in cultivated cacao plantations in Indonesia. The findings of this study suggest that midges alone were probably too rare to act as main or even sole pollinators of cacao in our study region. Potential additional pollinators would be small Diptera (e.g., Chloropidae and Phoridae) and Hymenoptera (e.g., Eulophidae and Platygastridae). Moreover, species composition of ceratopogonid midge assemblages in Bolivia differs from those in other studies. We thus did not find any of the ceratopogonid species reported by Winder (1997) in Brazil or by Sánchez *et al*. (2001) in Venezuela.

According to the hypothesis that some level of gene flow between wild and cultivated plants of *T. cacao* takes place, the results revealed bidirectional pollen flow between wild and cultivated trees. This was evident both for seeds from the paternity analysis but also for adult plants indicating that hybrids can establish under natural conditions. According to Arias and

Rieseberg (1994) and Fénart *et al.* (2007), in general, spatial separation among wild and cultivated forms alone is rarely enough to prevent gene flow. Indeed, considering the large pollen dispersal distances founded in *T. cacao*, physical distances of a few hundred meters as in this study cannot totally prevent pollen flow between both cacao forms. Wild populations are considered reservoirs of genetic diversity, but the studied wild populations showed lower genetic diversity than the crops. In this case, gene flow actually could increase the genetic diversity of wild cacao, although long-term consequences are currently unknown and need more study.

The populations of the three species of *Theobroma* displayed low, but significant small-scale spatial genetic structure, contrary to my hypothesis; I expected to find moderate SGS in populations of these species. Spatial genetic structure was more pronounced in *T. subincanum* than in the other species as evidenced by the low values of the *Sp* statistic in *T. speciosum* (0.004) and wild *T. cacao* (0.007) compared to *T. subincanum* (*Sp* = 0.048). Mean pollen dispersal distances in wild cacao (922 m) assessed with direct methods were larger than the gene dispersal distances inferred from SGS patterns (101 m), because the gene dispersal distance consider both pollen and seed dispersal, consequently takes in account the recrutment of seedlings in the forest. The largest gene dispersal distance was found in *T. speciosum* (208 - 310 m), suggesting more efficient seed and pollen dispersal.

The attraction of pollinators is important for the success of sexual reproduction in insect pollinated plants. In many plant species, the flower color, flower size, flowering intensity show differences in pollinator attraction, although in *Theobroma* apparently the differences in flower colors do not affect pollination between species (Santos *et al.*, 2012). In cacao I documented a wide range of insect floral visitors. In contrast, information about floral visitors in *T. speciosum* and *T. subincanum* lack, therefore a formal comparison among species is not possible. The available data on pollinators showed that Drosophilidae and

Phoridae flies are attracted to flowers of *T. speciosum*. More research on *T. speciosum* and *T. subincanum* is necessary to identify possible pollinator competition among those species. Young *et al*. (1984) and Erickson *et al*. (1986) documented differences in floral fragrance among species of *Theobroma*. Nevertheless, microclimatic conditions seem to influence pollination success more strongly than morphological differences.

GENERAL CONCLUSIONS

This study shows that the natural populations of *T. cacao* in northwestern Bolivia correspond to wild forms accordingly are genetically distinct from the cultivated trees, which confirm that Bolivia is part of the natural range distribution of *T. cacao*. Nevertheless, wild populations showed lower genetic diversity than the cultivated cacao, probably because cultivated forms come from a variety of origins (e.g., Costa Rica, Trinidad and Tobago). Surprisingly, and in contrast to early theories self-pollination takes place in *T. cacao* especially among cultivated trees, even though cross-pollination predominates. Most of pollination events documented in this study took place over distances of less than few hundred meters, but long pollen dispersal distances in wild and cultivated cacao were evidenced. Within wild populations, long distance pollen dispersal can play a major role in maintaining genetic connectivity. Pollination distances in cacao were larger than those typically reported in tropical understory tree species, considering the minute sizes of the pollinating midges, this finding reflects how dynamic and important the aerial plankton in transporting small pollinators is. Furthermore, this study revealed bidirectional pollen flow between wild and cultivated cacao. This was evident both for seeds from the paternity analysis but also for adult plants indicating that hybrids can establish under natural conditions. The relatively high pollen exchange from cultivated to wild cacao found compromises genetic identity of wild populations, calling for the protection of extensive natural forests tracts to protect wild cacao in Bolivia.

In respect to cacao pollinators, a wide range of flower visitors was documented as well as some differences between the visitors of wild and cultivated cacao. These findings suggest that Ceratopogonidae midges alone were probably too rare to act as main or even sole pollinators of cacao in our study region. Small Diptera (e.g., Chloropidae and Phoridae) and Hymenoptera (e.g., Eulophidae and Plagygastridae) could be potential additional pollinators.

My findings revealed differences among the three studied species in terms of gene dispersal distance and small-scale spatial genetic structure. *Theobroma speciosum* showed the largest gene dispersal distances whereas *T. subincanum* and *T. cacao* showed similar intermediate gene dispersal distances. The three species have rather low but significant small-scale spatial structure, which denotes relatively limited gene dispersal. *T. subincanum* showed the strongest SGS suggesting less efficient pollen and seed dispersal.

The findings of this study have implications for the formulation of conservation policies for the conservation of natural populations of *Theobroma*. The large pollen dispersal distances reported in cacao and the relatively high pollen exchange from cultivated to wild cacao pointed out the necessity for the protection of extensive natural forests tracts to protect the genetic identity of wild cacao in Bolivia and, in addition to promote genetic exchange among wild populations to maintain genetic variability of viable populations. Gene dispersal distances were intermediate for the three *Theobroma* species. The small-scale spatial genetic structure found in *Theobroma*, pointed out the importance of the protection of seed dispersal animals to maintain the genetic resources of this tropical tree genus.

Further research is needed on the long-term consequences of crop to wild gene flow in *T. cacao*. It would be interesting to compare Bolivian wild cacao populations with wild cacao from other countries in Latin America. The occurrence of self-pollination in *T.*

speciosum and *T. subincanum* needs to be studied. Very little is known about pollinators of *T. speciosum* and *T. subincanum*, it would be of interest also to study whether competition for pollinators between both species takes place, considering that both species commonly co-occur in the lowlands of Bolivia.

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ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisors Dr. Michael Kessler (Institute of Systematic Botany, University of Zurich, Switzerland), Prof. Dr. Teja Tscharntke (Agroecology, Department of Crop Sciences, Göttingen University) and Prof. Dr. Isabell Hensen (Geobotany and Botanical Garden, Institute of Biology Martin-Luther-University of Halle-Wittenberg, Germany) for their suggestions and all the support to make my PhD a productive and stimulating experience. Their immense knowledge and experience was fundamental for the development of this thesis. I want to specially thank Dr. Walter Durka (Department of Community Ecology, Helmholtz Centre for Environmental Research – UFZ, Germany), I have really benefited from his expertise and experience on population genetics.

My gratefulness to the German Academic Exchange Service (DAAD) for the scholarship granted. For the financial support I express my thanks to the German Science Foundation (HE 3041/17-1). I would also take the opportunity to thank Prof. Dr. Gansert for give me the opportunity to be part of the “Biological Diversity and Ecology” PhD Program.

I would like to express my genuine gratitude to Birgit Müller (T.A., Institute of Biology, Martin-Luther-University of Halle-Wittenberg, Germany) for teaching me molecular techniques, for her support during the lab work, and her friendship. I really learned a lot. Thanks to Sabrina Träger for helping me in the lab, many thanks for a very efficient work!

I would like to thank my friends and colleagues from Halle, Amira Apaza, Regine Brandt and Heidi Hirsch. In particular, thanks to Amira for helping me transporting my samples and for having me at her home; and to Regine thanks for helping me with the German translation of the abstract and for our friendly and pleasant work environment.

Thanks to the Agroecology group for the friendly work atmosphere, and for motivating me to improve my German. Especial thanks to Carmen Börschig, Ellie Vockenhuber, Christoph Rothenwöhrer, Kristin Krewenka, Vesna Gagic, Ines Vollhardt and, Barbara Scheid for their friendship. Thanks to Yann Clough and Janna Groenevald for providing me valuable ideas to planning the fieldwork and for sharing their experiences in cacao.

For assistance in the field, I would like to thank Rosenber Hurtado, Consuelo Campos, and the local assistants: Javier Navi, Carlos Ibaguari, Efraín Amutari, Wilson Amutari, and Leonardo Tube, also the people of Napashi, who kindly allowed me to do my research on their lands. In particular, thanks to Rosenber for his compromise with the project and his good ideas.

For insect's identification, thanks to the Ceratogonidae experts Dr. Gustavo Spinelli and Dr. Pablo Marino (National University of La Plata, Argentina). Thanks to Dr. Ines Vollhardt (University of Göttingen, Germany) for the identification of Hymenoptera and to Lic. Miguel Limachi for the identification of Diptera (Colección Boliviana de Fauna, Mayor de San Andrés University, Bolivia).

I thank the National Herbarium of Bolivia (LPB) for logistic support, especially to Dr. Stephan Beck. I also thank the Ministerio de Desarrollo Rural y Medio Ambiente – Dirección General de Biodiversidad y Áreas Protegidas in Bolivia for research permits, and the Department of Systematic Botany (Albrecht-von-Haller Institute for Plant Sciences, Göttingen University).

To Marcus who has been my support since the beginning of this journey, thanks for your ideas, the discussions, your time and for always being there for me. To Catalina who has been my inspiration, every single laugh from you makes this life better. Thanks for sharing this experience since you were in my belly. Love you both.

To my parents Betty and Jorge; and to my brothers David, Danny and Rodri thanks for all the love and support during my whole life. Mami thanks for taking care of my “gordita” while I was working on this thesis, I would not have finished it without your help.

My sincere gratitude to my parents in law Christa and Horst Schawe, thanks for helping me with the paperwork and for your support during the last part of this PhD.

I would like to thank my dear friends Karla Hernández, Aura Cárdenas and Mingwei Ren for their friendship and companionship. Love you guys.

Thanks to God for his blessings!

THESIS DECLARATION

Declaration of the author´s own contribution to manuscripts with multiple authors

The chapters II to IV are a series of manuscripts that have or will be submitted to peer-reviewed journals. I am the main author of all manuscripts presented in this thesis. I have personally collected and analyzed the data for all manuscripts, developed the main ideas presented in the manuscripts, written all manuscripts, made the tables, figures, and appendices, and contributed significantly to the sampling design. My supervisors and the co-authors laid out the study design and helped with analysis, discussions and writing.

Declaration plagiarism

I hereby confirm that I have written this doctoral thesis independently that I have not used other sources or facilities others than the ones mentioned, that I have not used unauthorised assistance and that I have not submitted this thesis previously in any form for another degree at any university or institution.

Göttingen, August 2013 Claudia Chumacero de Schawe

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