ACADÉMIE DE MONTPELLIER

AGRO M

ÉCOLE NATIONALE SUPÉRIEURE AGRONOMIQUE DE MONTPELLIER

THÈSE

Présentée à l'Ecole Nationale Supérieure Agronomique de Montpellier

pour obtenir le diplôme de doctorat

Formation Doctorale : Ressources Phytogénétiques et Interactions Biologiques
École Doctorale : Biologie des Systèmes intégrés, Agronomie-Environnement
Laboratoire d'Accueil : UMR 1097 Diversité et Génomes des Plantes Cultivées

Structure génétique, biochimique, morphologique et écologique de *Oenocarpus bataua* Mart. (Arecaceae) : perspectives pour la valorisation durable d'une ressource forestière néotropicale

Par

ROMMEL MONTUFAR GALARRAGA

Soutenue publiquement le 9 février 2007 devant le jury composé de:

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Daniel PRAT	Professeur, Université Lyon I	Rapporteur
Henri CARON	Ingénieur de Recherche, INRA Pierroton	Examinateur
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Serge HAMON	Directeur de Recherche, IRD Montpellier	Directeur de thèse


Tab. geograph. I

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I
INTRODUCTION

Oenocarpus bataua Mart.

I . INTRODUCTION

1.1 *La diversité biologique dans le milieu amazonien*

La forêt amazonienne représente une richesse considérable en terme de biodiversité. Par exemple dans le Parc National Yasuní, en Amazonie équatorienne, plus de 1104 espèces végétales ligneuses non lianescentes (dbh ≥ 10 mm) coexistent sur une parcelle de 25 ha (Valencia *et al*. 1994, Valencia *et al*. 2004). Cette diversité végétale est constituée par des espèces sauvages qui gardent encore l'essentiel de leur variabilité naturelle, laquelle résulte de multiples influences historiques (Haffer 1997, Colinvaux & De Oliveira 2001) et écologiques (Hubell 1979, Salo *et al*. 1986, Patton *et al*. 2000, Tuomisto *et al.* 2003).

Plusieurs modèles allopatriques ou de vicariance biogéographique[1] ont été proposés concernant les processus de genèse et de maintien d'une telle diversité végétale dans les écosystèmes amazoniens. Les premières hypothèses proposées pour expliquer les disjonctions biogéographiques dans le bassin Amazonien ont été basées sur les patrons de distribution des palmiers. Spruce (1871) suggère cinq divisions phytogeographiques dans l'Amazonie (Tableau 1). Le modèle de Spruce prend en compte l'histoire géologique comme la majeure force structurant les distributions biogéographiques des palmiers. Par exemple, l'absence d'espèces de palmiers endémiques de l'Amazonie centrale a été expliquée pour la présence d'un ancien lac ou d'une série de lacs qui couvraient toute cette région. Les travaux ultérieurs basés sur la distribution des palmiers, comme ceux de Drude (1882) et particulièrement Barbosa Rodriguez (1903) sont en accord avec le modèle de Spruce (Tableau1).

D'autres théories, basées sur des données géologiques suggèrent que cette biodiversité a été structurée par des événements géologiques à grande échelle qui ont favorisé la spéciation allopatrique. De tels macro-événements incluent l'orogenèse andine (Gentry 1982, Brumfield & Capparella 1996), des transgressions marines (Nores 1999), des formations lacustres (Tertiary lake, Frailey *et al*. 1988), les variations du cours des rivières (Salo *et al*. 1986, Räsänen *et al.* 1987, Salo 1988, Räsänen *et al*. 1990) et l'évolution de la géomorphologie amazonienne (Arch theory, Rasanen *et al*. 1990). Les grandes rivières de l'Amazonie sont également considérées comme des barrières naturelles au flux génétique

[1] Les peuplements spontanés divisés pour une barrière naturelle

des populations de plantes et d'animaux (Wallace 1849, Caparella 1988, Ayres 1992). L'hypothèse de perturbation-vicariance est quant à elle basée sur les changements climatiques pendant le Pléistocène (la théorie des refuges proposée pour Haffer 1969, Vuilleumier 1971, Prance 1982; Distubance-Vicariance hypothesis, Colinvaux & De Oliveira 2001). En particulier, la théorie des refuges de Haffer a dominé la discussion sur la biogéographie de l'Amazonie durant les dernières décénies. La théorie des refuges suggère que la biodiversité amazonienne s'est lentement mise en place au cours du Tertiaire et a pu se maintenir durant les phases froides et sèches du Pléistocène au niveau de refuges forestiers assez largement répartis, permettant une simple recolonisation de l'ensemble de la région au Quaternaire tardif (Haffer 1969, Prance 1982, Hooghiemstra et van der Hammen 1998).

Tableau 1. Les modèles phytogeographiques de l'Amazonie basés sur la distribution de palmiers. Les flèches montrent l'équivalence entre modèles.

	Spruce 1871		Drude 1882		Barbosa Rodrigues 1903
1	Coast or Submaritime	→	*Littorales*	→	*Littoraliae*
2	Granite	→	Boreali occidentales	→	*Cataractae borealiae*
3	Diamond	→	Australi Occidentales	→	*Cataractae australiae*
4	Amazon	→	Centrales	→	Planae/eastern
5	Subandine	→		→	Planae/western

D'autres théories abordent la question de la biodiversité sous l'angle des mécanismes de coexistence d'espèces. L'effet des disséminateurs des graines (Gentry 1983), la dynamique forestière (Dumont *et al.* 1990, Kahn 1996), la concurrence entre espèces (Hubbell 2001), l' hétérogénéité de l'environment (Tilman 1994), la spécialisation d'habitat (Ashton 1969, Gentry 1989, Tuomisto & Poulsen 1996), l'herbivorie (Leigh

1990), la dynamique forestière (Connell 1979, Salo *et al.* 1986), entre autres, ont été proposés comme hypothèses pour expliquer la persistance de la diversité biologique aux échelles locale et régionale. Selon ces hypothèses, l'interruption du flux génique par des mécanismes écologiques est suffisante pour favoriser la spéciation.

Les palmiers constituent un élément marquant de la forêt néotropicale. Le genre de palmiers *Oenocarpus,* et particulièrement l'espèce *O. bataua* constitue un modèle intéressant pour caractériser la structure éco-géographique de la diversité génétique. Cette espèce est constituée d'un ensemble de populations spontanées plus ou moins différenciées morphologiquement et écologiquement, abondant dans les écosystèmes forestiers et largement distribué dans la région. L'étude de la dynamique de la diversité génétique de *O. bataua* offre un éclairage nouveau sur la variabilité de l'espèce et constitue également un apport à la taxinomie de l'espèce. L'histoire taxinomique du genre *Oenocarpus* et de l'espèce *O. bataua* restant encore conflictuelle, la recherche sur la variabilité génétique, biochimique, écologique et morphologique fournit des arguments en faveur ou en défaveur des diverses interprétations taxinomiques existantes.

1.2 *Description Botanique de Oenocarpus bataua Martius*

Le genre *Oenocarpus* appartient à la tribu des *Euterpeae*, endémique néotropicale (Dransfield *et al.* 2005), dont les affinités sont encore mal établies, mais qui semble avoir un lien avec la tribu paléotropicale des *Areceae* plutôt qu'avec d'autres groupes néotropicaux (Hahn 2002a). Les *Euterpeae* comprennent cinq genres (*Euterpe*, *Hyospathe*, *Prestoea*, *Neonicholsonia*, *Oenocarpus*) qui habitent les régions tropicales et sub-tropicales de l'Amérique. Les genres regroupés dans la tribu *Euterpeae* partagent les caractères suivants (a) feuilles pennées ou à nervation pennée ; (b) une inflorescence à un ordre de ramification ou en épi; (c) un gynécée uniloculaire du fait de l'avortement précoce de deux des trois carpelles (pseudomonomérie) ; (d) un endocarpe mince et fragile (Uhl & Dransfield 1987).

Figure 1. *Oenocarpus bataua*, (A) Individus adultes dans les environs de la localité de Rioja (Pérou); (B) Infructescence de *O. bataua* var. *oligocarpa* dans la Réserve Naturelle des Nouragues (Camp Aratai, Guyane française); (C) Inflorescence de O. bataua var. bataua dans les environs de Tarapoto (Pérou); (D) Fruits mûrs.

Le genre de palmiers *Oenocarpus* est largement répandu dans les forêts humides de l'Amérique du Sud tropicale. Ce genre comprend neuf espèces (Henderson 1995), toutes présentes dans la région amazonienne. Le genre *Oenocarpus* est constitué par des palmiers de grande taille (*Oenocarpus bataua*, *O. bacaba*), ou des palmiers de sous-bois dont certains de taille modeste (*O. simplex*, *O. circuntextus*). L'inflorescence hippuriforme (en forme de queue-de-cheval) est le caractère morphologique le plus remarquable du genre.

L'espèce *Oenocarpus bataua* est très abondante dans les écosystèmes forestiers humides, elle se développe dans la canopée ou sous-canopée de la forêt primaire et persiste dans les milieux anthropisés en raison de sa résistance aux conditions exposées, aux brûlis, à la coupe (tronc très dur et irrigué de vaisseaux dans toute son épaisseur, Kahn & Granville 1992). Ces palmiers peuvent aussi êtres intentionnellement conservés pour leurs usages. *Oenocarpus bataua* est largement distribué dans la région amazonienne (Kahn & Castro 1987, Balslev & Barfod 1987, Braun & Delascio 1987, Kahn & Mejia 1990, Moraes 1996, Kahn 1997, Granville 2002, Lorenzi *et al.* 2004). Au nord, il atteint les *llanos* de l'Orinoco, la forêt humide de la côte caraïbe, le foret guyanaise jusqu'à 700 m d'altitude, l'île de Trinidad et atteint tout juste l'isthme de Panama. Au sud, l'espèce atteint le c*errado* brésilien, et la région du *Beni* (Bolivie). À l'ouest, *O. bataua* pousse sur les pentes andines jusqu'à 1000-1400 m d'altitude et atteint la côte pacifique du nord-ouest de l'Amérique du Sud (région du *Chocó*). À l'est, *O. bataua* a été collecté sur la côte Atlantique jusque sur l'île de Marajó dans l'embouchure de l'Amazone (Henderson *et al*. 1991).

Oenocarpus bataua est un palmier arborescent monocaule, monoïque, allogame. Le stipe atteint 12-25 m de hauteur avec un diamètre de 15-45 cm. Les feuilles de 10-12 m de long sont érigées et pennées (80-120 paires de pennes). Les pennes (taille maximale: 100-180 cm x 6-12 cm), sont fortement nervurées, disposées régulièrement dans un plan, de couleur vert glauque et couvertes d'une épaisse couche de cire et de trichomes à la face inférieure. La gaine de la feuille présente des fibres linéaires noires, rigides et longues (1-2 m). Ce palmier produit de grandes inflorescences hippuriformes protandres et infrafoliaires (Borgtoft Pedersen & Balslev 1993). Les inflorescences sont pourvues d'un pédoncule court et robuste prolongé par un rachis conique de 25-50 cm de longueur d'où naissent 100 à 300 rameaux florifères de 0.7-1.2 m de longueur. Les rameaux florifères sont de couleur jaune pâle lors de la floraison et virent au –marron rougeâtre en

fructification. La partie proximale de chaque rameau florifère porte des triades composées d'une fleur femelle centrale et deux fleurs mâles latérales tandis que la partie distale du rameau florifère ne porte que des paires de fleurs mâles. Les fruits sont ellipsoïdes, de couleur violacé foncée à maturité, de 2.5-4.5 cm de longueur et 2.2-2.5 cm de diamètre. Le mésocarpe charnu est oléagineux. Un arbre adulte peut produire plus de 100 kg de fruits par an (Miller 2002, Figure 1).

Oenocarpus bataua est connu pour s'hybrider avec les autres espèces du genre comme *O. bacaba* et *O. mapora*, produisant des plantes stériles (Balick 1981). Plusieurs espèces décrites soit dans le genre *Oenocarpus* soit dans le genre synonyme *Jessenia* ont été ensuite incluses dans l'espèce *O. bataua*: *J. polycarpa* H. Karst. du Chocó Colombien, *J. weberbaueri* Burret du piémont andin péruvien, *J. oligocarpa* Griseb. & Wendl. de Trinidad (Balick 1986).

L'écologie de *Oenocarpus bataua* est variable suivant les régions. Dans la périphérie du bassin Amazonien (Haute Amazonie), il pousse sur terres fermes (Montufar 1999) et monte dans les Andes, poussant sur fortes pentes jusqu'à 1350 m d'altitude (Borgtoft & Balslev 1993). En basse Amazonie, il pousse uniquement sur sols hydromorphes, fréquemment associé à un autre palmier de grande taille, *Mauritia flexuosa* (Kahn *et al.* 1988, Kahn & Mejia 1990). *O. bataua* est une espèce clé en raison de ses multiples interactions avec les disperseurs et consommateurs de pollen et de fruits (Snow & Snow 1978, Sist & Puig 1987, Link & Di Fiore 2006). De plus, il est un des plus grands producteurs de matière organique dans les écosystèmes forestiers amazoniens (Kahn & Granville 1992).

1.3 *Botanique Economique*

Oenocarpus bataua –localement appelé pataua (Brésil), ungurahui (Pérou), mil pesos (Colombie), chapil, ungurahua (Equateur), patawa (Guyane française), majo (Bolivie), palma seje (Venezuela)– est un palmier important pour les populations locales en raison de ses usages alimentaires, médicinaux, rituels, et comme source de matériaux de tressage, de construction et pour la confection d'objets (Balick 1986, Macia 2004). Une vaste bibliographie sur l'ethnobotanique et la botanique économique de *O. bataua* se trouve dans Cavalcante (1974), Balick (1981, 1984, 1986), Balick & Gershoff (1981), Borgtoft

Pedersen & Balslev (1993), Kahn (1993), Andrade Miranda (2001), Macia (2004) entre autres.

L'usage le plus important de *O. bataua* est celui de ses fruits oléagineux. Les fruits sont utilisés principalement (i) pour l'obtention de l'huile et (ii) la préparation d'une boisson nutritive (appelée « chicha » en Equateur, «agua de seje » en Venezuela). L'huile est extraite du mésocarpe du fruit (qui en contient de 12 à 18 %) de façon traditionnelle. L'extraction se fait par macération des fruits, puis séparation de la pulpe des graines manuellement; enfin l'huile est purifiée par chauffage et filtration (Balick 1981). Pour l'élaboration des boisson nutritives, les fruits sont d'abord mis à bouillir dans un grand récipient, puis la pulpe est séparée des graines et de la masse de fibres qui se déposent au fond du récipient. La pulpe est pressée manuellement afin de la débarrasser de l'huile. La boisson qui en résulte est filtrée. Cette boisson au goût et couleur chocolaté est largement vendue sur les grands marchés de la région amazonienne comme Belém, Iquitos, Manaus, Tarapoto, Pucallpa, Cayenne, mais aussi dans les petits villages. De plus, quand les fruits sont mûrs, les gens de la région les consomment directement.

Aujourd'hui, la consommation de l'huile de *Oenocarpus bataua* a pratiquement disparu et son importance commerciale en Amazonie est minime. En 1974, Cavalcante note la disparition de l'huile de *O. bataua* du marché de Belém (Brésil). Une situation similaire est observée dans d'autres pays de la région . Les causes possibles sont : (1) l'obtention d'huile de *O. bataua* est un processus laborieux, en commençant par la récolte dans les populations naturelles et le transport des fruits à dos d'homme, (2) l'extraction manuelle ne favorise pas la production à grand échelle, (3) la production de l'huile de *O. bataua* est basée sur l'exploitation des populations sauvages qui n'assurent pas de récoltes continues, et (4) la concurrence des huiles commerciales comme l'huile de soja et de palme (*Elaeis guineensis*), entre autres. Par conséquent, la production d'huile de *O. bataua* est faible et coûteuse.

En Equateur, l'huile de *O. bataua* est plus connue pour ses propriétés médicinales que nutritionnelles. Cette huile est prisée comme un tonifiant capillaire et largement vendue dans les boutiques de phytothérapie des grandes villes d'Equateur ainsi que dans toute la région amazonienne du pays. Enfin, une petite industrie s'est développée, basée sur

l'extraction de la pulpe de *O. bataua,* pour l'élaboration des glaces dans différents pays, en particulier le Pérou, le Brésil et la Guyane française.

1.4 *Perspective Economiques de Oenocarpus bataua*

Oenocarpus bataua est classé par la FAO parmi les espèces végétales à potentiel de domestication (Balick 1988). Plusieurs études éthnobotaniques et analyses nutritionnelles des fruits montrent le potentiel économique de cette espèce. La principale caractéristique biochimique de l'huile de *O. bataua* est la prédominance des acides gras insaturés. La composition en acides gras de cette huile est proche de celle de l'huile d'olive, c'est-à-dire, riche en acide gras oléique (jusqu'à 80%), avec une faible proportion d'acides gras saturés comme les acides palmitique (13-15%) et laurique (< 1% ; Lleras & Coradin 1988, Lubrano & Robin 1997, Balick & Gershoff 1981, Aleman *et al.* 2002). La faible quantité d'acide linoléique (2%) est la seule différence avec l'huile d'olive (5-15% dans l'huile d'olive, Ucciani 1995). A niveau des caractéristiques physiques (indice de réfraction, taux de saponification), cette huile est également très similaire à l'huile d'olive (Pesce 1985). L'huile de *O. bataua* est riche en aminoacides (Balick & Gershoff 1981). Dans une région comme l'Amazonie, où la majorité de la population souffre de malnutrition, avec en particulier un déficit protéique et une forte consommation d'acides gras saturés, la production et la consommation d'huile de *O. bataua* est une option recommandable et susceptible de générer une activité économique substantielle.

L'exploitation actuelle des fruits de *Oenocarpus bataua* relève d'une forme destructrice d'extractivisme, lequel met en danger la survie des peuplements spontanés. Les populations qui habitent la région abattent les arbres adultes dans le but d'atteindre leurs fruits situés à 12-15 m de hauteur ou plus (voir Kahn 1991). Cette activité est conduite aux abords des villages pendant la période de fructification. Aujourd'hui, les peuplements spontanés présentant une grande densité d'adultes sont de plus en plus difficiles à trouver dans les lieux habitées de l'Amazonie. Par exemple, dans la localité de Jenaro Herrera au Pérou (basin de l'Ucayali), à l'exception de quelques populations protégées dans des réserves privées, la population d'adultes la plus proche du village est à deux heures de marche.

Bien que le potentiel économique de *O. bataua* ait été principalement envisagé en relation à l'extraction de l'huile du mésocarpe, la graine constitue également une riche source d'acides gras non exploitée. À la différence du mésocarpe, la graine de *O. bataua* est riche en acide laurique (42%) et oleique (16% ; Salazar *et al.* 2004). L'huile de la graine de *O. bataua* pourrait être un substitut des huiles saturées extraites de *Cocos nucifera* et de *Elaeis guiannensis* pour les usages industriels.

1.5 *Objectifs de la thèse*

Cette thèse a pour but d'étudier la variabilité moléculaire, biochimique, morphologique et écologique de *Oenocarpus bataua* dans la forêt tropicale de l'Amérique du Sud. Plusieurs interpretations taxinomiques du genre *Oenocarpus* et de l'espèce *O. bataua* seront testées à partir de donnes moléculaires, écologiques, morphologiques et biochimiques. Les résultats seront discutés dans le cadre des théories de diversification dans l'Amazonie. Notre approche est structurée en trois niveaux d'organisation:

1.5.1 Structure phylogénétique du genre *Oenocarpus* et de la tribu *Euterpeae*.

Les relations évolutives entre espèces sont importantes à considérer pour la gestion de ressources génétiques; plus les taxons sont proches génétiquement, plus ils ont de chances de pouvoir se croiser. Le genre *Oenocarpus* a été relativement peu étudié, en particulier en ce qui concerne sa taxinomie, qui reste très imprécise. En 1823, Martius (voir Balick 1986) décrit le genre *Oenocarpus* avec cinq espèces, dont *O. bataua* Martius. Karsten (1857) décrit le genre *Jessenia* avec le type *J. polycarpa*. Dans ce travail, Karsten ne fait pas aucune mention de l'affinité taxinomique entre *Jessenia* et *Oenocapus*. Burret (1928) propose une nouvelle combinaison taxinomique. Il transfère l'espèce *O. bataua* dans le genre *Jessenia* (*Jessenia bataua* (Martius) Burret). Sur la base d'un important travail en herbier et de récoltes sur le terrain, Balick (1986), publie un traitement taxinomique du complexe *Oenocarpus-Jessenia*. Balick donne des éléments morphologiques et biochimiques en faveur de la séparation taxinomique entre le genre *Oenocarpus* et le genre *Jessenia*, ce dernier avec une seule espèce (*J. bataua*) et deux sous-espèces (*bataua* et *oligocarpa*). Cependant, une étude phylogénétique récente de la tribu Euterpeae, basée sur les caractères morphologiques et anatomiques (Henderson 1999), ainsi que la découverte d'une espèces de *Oenocarpus* (*O. makeru*) ayant certains caractères

morphologiques de *Jessenia* (Bernal *et al.* 1991), ont mis en doute le statut taxinomique du genre *Jessenia* proposée par Balick (1986).

Deux interprétations taxinomiques résument l'histoire conflictuelle des genres *Oenocarpus* et *Jessenia*. La première considère *Jessenia* comme un genre proche mais distinct de *Oenocarpus* (Burret 1928, Balick 1986) et incluant une seule espèce, *Jessenia bataua*, avec deux sous-espèces (*bataua* et *oligocarpa*). Le deuxième ne considère pas *Jessenia* comme un genre différent de *Oenocarpus*. L'espèce *J. bataua* est mise en synonymie de *O. bataua*, avec deux variétés, var. *bataua* et var. *oligocarpa* (Henderson 1995). Bien qu'actuellement la proposition de Henderson soit largement acceptée, nous avons voulu tester le statut de *Jessenia* par rapport à *Oenocarpus sensu stricto* en réalisant une phylogénie de la tribu *Euterpeae* incluant un échantillonnage représentatif du groupe *Oenocarpus/Jessenia*, basée sur des séquences d'ADN chloroplastique.

Au sein des *Euterpeae*, *Oenocarpus* et *Jessenia* se distinguent par leur inflorescence hippuriforme (en queue-de-cheval). *Jessenia* se distinguent de *Oenocarpus* par ses graines à albumen ruminé (homogène chez *Oenocarpus*), ses fleurs mâles à 8-19 étamines (6 chez *Oenocarpus*), ses étamines à filament non réfléchi apicalement (réfléchi chez *Oenocarpus*) et par la présence de trichomes à la face inférieur des feuilles (cire seulement chez *Oenocarpus*). La seule analyse phylogénétique des *Euterpeae* existante est celle de Henderson (1999), basée sur les caractères morphologiques et anatomiques. *Jessenia* apparaît dans cette analyse inclus dans le genre *Oenocarpus*, formant un clade avec *Oenocarpus simplex* et *O. circumtextus*, et non à proximité de *O. minor*, *O. bacaba* et *O. mapora* qui semblaient avoir des affinités avec *Jessenia* sur la base des hybridations ou de caractères similaires. Il se peut que ce regroupement résulte d'un artefact analytique d'attraction des longues branches, en raison des caractères fortement dérivés des trois espèces *O. bataua*, *O. simplex* et *O. circumtextus*. Cette étude cladistique apparaît comme le seul argument qui soutient l'inclusion de *Jessenia* dans le genre *Oenocarpus*. Il est donc nécessaire de réaliser une phylogénie moléculaire des *Euterpeae*, afin de clarifier ces relations phylogénétiques qui ne peuvent être établies clairement sur la base des seuls caractères morphologiques.

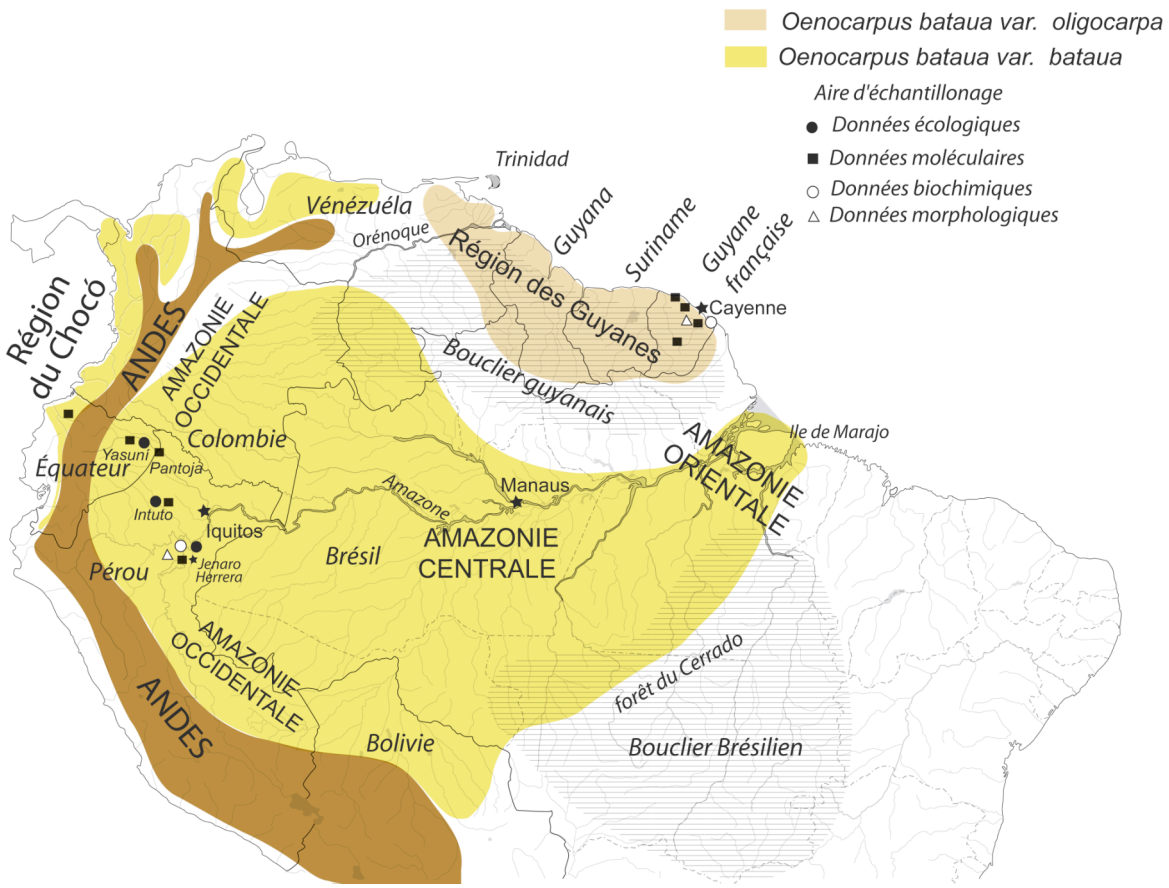


Figure 2. Aire de distribution de *Oenocarpus bataua* dans l'Amérique du Sud.

Ici, nous proposons d'établir les relations phylogénétiques entre *Jessenia* et *Oenocarpus* à partir des séquences nucléotidiques d'espaceurs intergéniques du chloroplaste (cpADN). Nous avons travaillé sur les régions *trn*D-*trn*T, *trn*Q-*rps*16, *trn*S-*trn*fM et *psb*C-*trn*S publiées par Desmesure *et al.* (1995) et Hahn (2002a) et largement utilisées pour la phylogénie des palmiers (Hahn 2002a,b). Ces régions ont été surtout utilisées au niveau générique, car elles se montrent moins informatives au niveau interspécifique (voir Baker *et al.* 1999). Toutefois, une étude phylogénétique dans le genre *Bactris* (Couvreur *et al.* sous presse), a montré que l'ADN chloroplastique est encore informatif pour établir les relations phylogénétiques au niveau d'espèces, surtout si l'on prend en compte non seulement les données de substitutions, mais également d'indels, microsatellites et inversions.

Une analyse cladistique sera construite en identifiant les mutations successives et en minimisant le nombre d'évènements (principe de Parcimonie) .

1.5.2 Structure infraspécifique de *Oenocarpus bataua*

Structuration génétique, biochimique et morphologique entre Oenocarpus bataua var. *bataua et O. bataua* var. *oligocarpa*

Le fait que le complexe *Oenocarpus bataua* soit actuellement considéré comme une seule espèce (avec de nombreux synonymes) résulte d'une tendance au regroupement des taxons apparue dans les monographies récentes des palmiers néotropicaux. Cette conception appelée « lumping taxonique » masque en fait la diversité réelle des groupes concernés. De plus, ces monographies sont basées sur l'analyse d'herbiers essentiellement, une approche insuffisante pour cerner la réalité biologique d'espèces largement répandues dans toute la région néotropicale. Dans le cas de *O. bataua*, les populations provenant des Guyanes ont été au départ considérées comme une espèce distincte, *Oenocarpus oligocarpa* ou *Jessenia oligocarpa* (Wessels Boer 1965, Grisebach & Wendland 1864 in Balick 1986). Des études postérieures, basées sur des données morphologiques (Balick 1986, Henderson 1995) ont réduit cette espèce au rang de sous-espèce ou variété de *Oenocarpus bataua*, décrit de l'Amazonie centrale et occidentale. Plusieurs noms botaniques comme *O. polycarpa*, *O. seje*, *Jessenia weberbaueri* corrrespondent clairement à *O. bataua* var. *bataua* et sont acceptés comme synonymes.

La variation morphologique parmi des deux variétés *bataua* et *oligocarpa* demeure encore peu étudiée. La variété *bataua* se distingue de la variété *oligocarpa* par le positionnement des paires de fleurs mâles sur les rameaux florifères (limitée à la zone apicale chez var. *oligocarpa*), le nombre et la morphologie des rameaux florifères (plus nombreux et plus fins chez var. *oligocarpa*), le nombre d'étamines (7-11 chez *var. oligocarpa*, 7-20 chez *var. bataua*), la taille des fleurs femelles (4-5 mm chez *var. oligocarpa*, 5-7 chez var. *bataua*) et le type d'indûment sur la face abaxiale des pennes (couleur blanchâtre chez *var. oligocarpa*, couleur grisâtre chez *var. bataua*) .

Dans ce chapitre, nous rechercherons donc les discontinuités génétiques, morphologiques et biochimiques pouvant correspondre à cette différentiation taxinomique au sein de *Oenocarpus bataua*. Si les deux variétés s'avèrent très différenciées sur les plans génétique, morphologique et biochimique, il faudra probablement considérer un traitement plurispécifique en réhabilitant des espèces anciennement décrites. La mise en évidence de la structure taxinomique au sein de *Oenocarpus* fournira des éléments de compréhension sur la dynamique de la diversité d'un groupe largement réparti en Amérique du Sud et donc sur la biogéographie régionale. Enfin, la connaissance de la structure génétique de *O. bataua* sera importante pour sa valorisation agricole et agronomique, dans la mesure où cette connaissance pourra servir de base à l'amélioration génétique de la plante. Ce chapitre est structuré en trois parties :

(*i*) *Analyse de la structuration de la variabilité génétique chez Oenocarpus bataua à l'aide de marqueurs AFLP.*

Les marqueurs dominants, comme les AFLP (Vos *et al.* 1995), sont largement utilisés chez les espèces à fort taux d'hétérozygotie comme les arbres forestiers (Prat *et al.* 2006). Nous avons utilisé les AFLP pour décrire la variabilité génétique de plusieurs populations de *O. bataua* provenant de Guyane (var. *oligocarpa*), de l'Amazonie occidentale et la forêt du Chocó (var. *bataua* ; Figure 2). Une approche classique d'analyse des données a été menée, incluant une Analyse Moléculaire de Variance (AMOVA, Excoffier *et al.* 1992) des analyses multivariées (Analyses en Coordonnées Principales, Legendre & Legendre 1998), et des dendrogrammes d'ordination (UPGMA). De plus, un polymorphisme d'inversion détecté dans la région chloroplastique *Psb*C-*trn*S a été utilisée comme un marqueur moléculaire pour différencier les variétés *bataua* et *oligocarpa*.

(ii) *Analyse de la structuration de la variabilité biochimique de Oenocarpus bataua au moyen de l'identification et de la quantification des acides gras du mésocarpe*

L'approche chimiotaxinomique a été peu appliquée à la taxinomie des palmiers néotropicaux. Balick (1986) a réalisé le seul travail avec des marqueurs biochimiques (flavonoïdes) appliqué à la taxinomie du genre *Oenocarpus*. Les fruits de palmiers sont riches en acides gras et ceux-ci pourraient être utilisés comme marqueurs pour étudier la taxinomie et la variabilité biochimique chez les palmiers. De plus, la composition en acides gras des fruits constitue l'intérêt agronomique de *O. bataua*.

En raison du manque de renseignement sur la composition en acides gras des fruits de *O. bataua*, nous avons décidé d'effectuer une analyse pour décrire la composition en acides gras de 38 échantillons de mésocarpe de ce palmier en utilisant la méthode de la chromatographie en phase gazeuse. L'échantillonnage comprend les deux variétés *bataua* et *oligocarpa* (Figure 2). Une analyse classique des données chimiotaxinomiques a été réalisée (Analyses en Composantes Principales et Analyse de Variance).

(iii) *Analyse et structuration de la variabilité morphologique de Oenocarpus bataua*

En raison de difficultés techniques, l'obtention des données morphologiques de *Oenocarpus bataua* dans la forêt amazonienne a été limitée à une étude préliminaire avec sept caractères végétatifs et huit caractères reproducteurs. Les différences entre variétés ont été évaluées au moyen d'une Analyse de Variance.

1.5.3. Structure populationnelle et différentiation inter-populationnelle

La structuration génétique et écologique des populations de Oenocarpus bataua dans l'Amazonie occidentale

Oenocarpus bataua est une espèce forestière à port arborescent, monoïque et allogame (Balick 1986), particulièrement importante sur le plan écologique et biogéographique dans la mesure où : (*i*) Il est largement répandu dans l'Amazonie, et particulièrement dans l'Amazonie occidentale il est très abondant (Kahn 1988, Montúfar & Pintaud 2006), (*ii*) il montre deux écotypes en relation à l'hydromorphie du terrain (forêts de *terra firme* sur sols bien drainées *vs.* forêts de bas-fonds et sur des sables blancs hydromorphes). Cette

différentiation écologique n'est pas associée à la variation morphologique (Kahn & de Granville 1992, Borgtoft Pedersen & Balslev 1993). Il sera donc intéressant d'observer comment est structurée la diversité génétique entre ces écosystèmes.
Ce chapitre est divisé en deux parties:

(*i*) *Variation écologique des populations de Oenocarpus bataua dans l'Amazonie occidentale.*

L'écologie de *O. bataua* dans l'Amazonie occidentale est incomplètement connue. Études botaniques dans la périphérie du bassin amazonien (Amazonie équatorienne) rapportant la présence de *O. bataua* dans les forets de *terra firme* sur sols bien drainée (Montufar 1999, Svenning 1999, Romero-Saltos *et al.* 2001, Duque *et al.* 2002). Par contra, les études écologiques en basse Amazonie occidentale et centrale montrent l'association de *O. bataua* à des sols hydromorphes (Kahn & Castro 1985, Kahn 1988, 1991; Kahn & Mejia 1990). Peres (1994) documente la présence de peuplements de *O. bataua* dans les forêts de *terra firme* de l'Amazonie centrale. Cependant, de vastes espaces de l'Amazonie occidentale demeurent inexplorés et par conséquent les données sur les patrons de variation de l'écologie de l'espèce sont encore fragmentaires. À l'aide d'un ensemble de transects réalisés dans trois localités de l'Amazonie occidentale (Intuto, Jenaro Herrera et Yasuni; Figure 2) nous chercherons à quantifier ces différences écologiques à l'aide d'outils statistiques appropriés (test de Mantel, régression logistique).

(*ii*) Construction d'une banque enrichie en séquences microsatellites $(GA)_n$ du palmier *Oenocarpus bataua* et génotypage sur un échantillonnage des trois populations ouest-amazoniennes.

En raison du faible taux de transfert des microsatellites isolées d'espèces et de genres proches (*O. bacaba*, Lepsch-Cunha *et al.* 2003; *Euterpe edulis*, Gaiotto *et al.* 2001) vers notre espèce d'interêt, nous avons construit la première banque enrichie en microsatellites $(GA)_n$ pour cette espèce. Vingt-trois locus microsatellites ont été isolés de *O. bataua* var. *bataua* avec la méthode de Billotte *et al.* (1999). Ces microsatellites sont très polymorphes, avec un taux élevé de transfert aux autres genres d'*Euterpeae*.

La structure génétique de trois populations de *Oenocarpus bataua* a été étudiée avec huit marqueurs microsatellites (Figure 2). Le rapport entre la structure génétique et la variabilité écologique de ces trois populations est aussi abordé. En plus des statistiques F et d'une Analyse Moléculaire de Variance, la recherche d'une structure génétique parmi les échantillons a été effectuée avec une méthode bayesienne implémentée dans le logiciel STRUCTURE (Pritchard *et al.* 2000).

1.6. Matériel et Aires d'études

Oenocarpus bataua est largement répandu dans la forêt tropicale de l'Amérique de Sud. Il existe une différentiation morphologique nette entre les populations d'Amazonie occidentale et celles des Guyanes, lesquelles constituent les variétés botaniques *bataua* et *oligocarpa*, respectivement (Figure 2). Pour cette raison, ces deux régions biogéographiques de l'Amérique du Sud ont été choisies pour cette recherche. De plus, nous avons inclus un échantillonnage provenant de la forêt de la côte Pacifique du nord-ouest de l'Amérique du Sud (forêt du Chocó), une région physiquement isolée du basin Amazonien par la cordillère des Andes. Ces populations situées à l'ouest des Andes appartiennent à la variété *bataua*. À l'est des Andes et sur la côte Caraïbe, les limites de distribution des deux variétés ne sont pas clairement établies.

Dans chaque région considérée, nous avons réalisé nos études sur plusieurs sites. La forêt du Chocó équatorien a été étudiée dans les provinces de Pichincha et Esmeraldas, située dans la région nord-occidentale de l'Equateur. Dans cette région, nous avons effectué un échantillonnage d'individus pour analyse génétique avec la méthode AFLP. Les populations de l'Amazonie occidentale étudiées se situent principalement au Pérou, dans les localités de Jenaro Herrera (bassin de l'Ucayali), Intuto (bassin du Rio Tigre), Pantoja (bassin du haut Napo), toutes trois dans la région Loreto. Dans ces sites ont été réalisés les échantillonnages pour analyse génétique utilisant les marqueurs microsatellites et les études sur l'écologie des populations (sauf Pantoja car nous avons utilisé nos données écologiques antérieurement acquises sur le site équatorien adjacent de Yasuní). Des analyses morphologiques et biochimiques ont été conduites principalement sur les échantillons de Jenaro Herrera. Enfin, quelques données additionnelles ont été incorporées (AFLP, séquençage de régions chloroplastiques), provenant d'autres sites du Pérou (Pasco, San Martin, Amazonas) et de l'Equateur (Napo, Pastaza). La forêt de Guyane

française a été considérée comme une zone représentative de la forêt du bouclier guyanais, et notre échantillonnage pour analyses AFLP, séquences chloroplastiques, morphologie et biochimie des lipides dans cette région a été réalisé dans les localités côtières comme Regina, les environs de Cayenne, Kourou, et également dans la localité de Saül dans le centre de la Guyane.

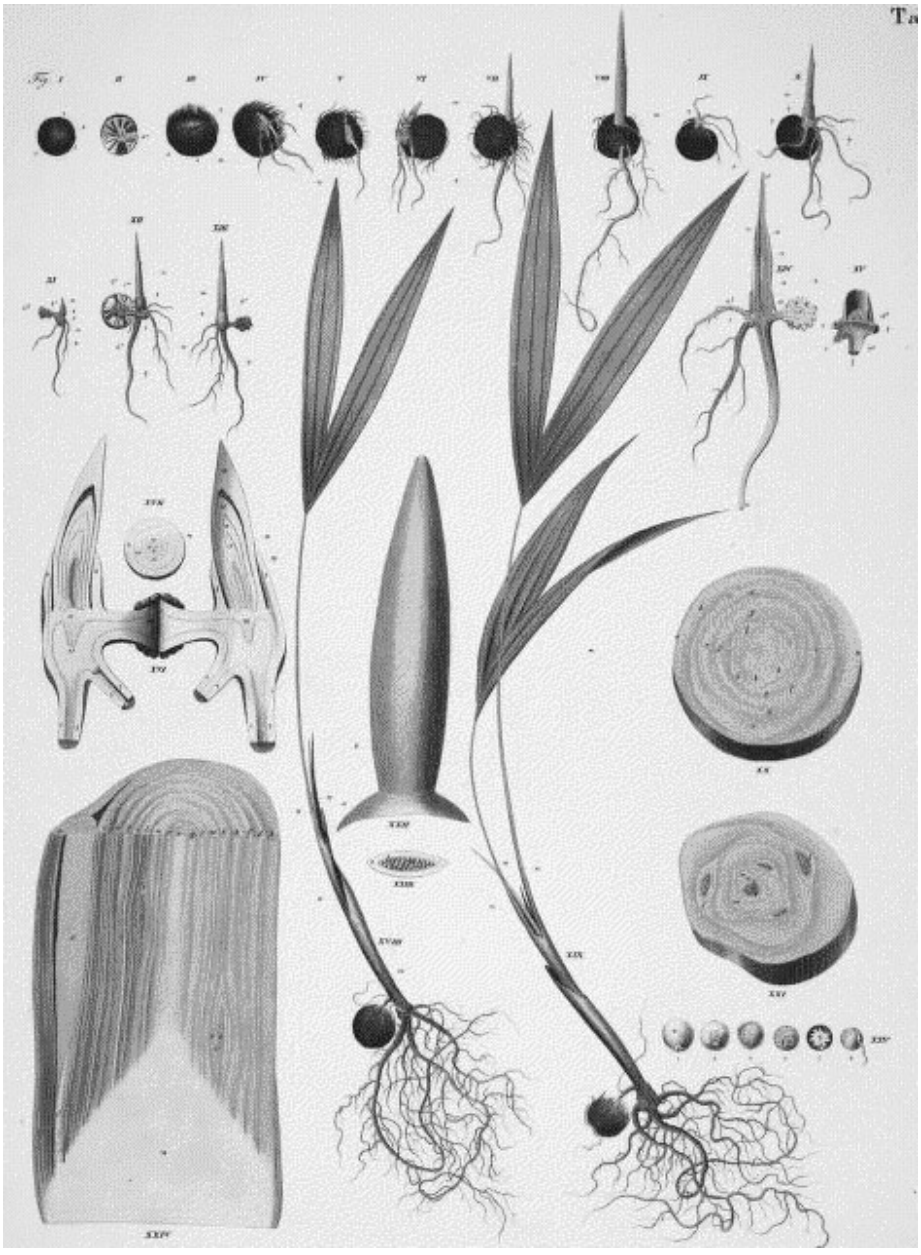
Synopsis des données acquises

(1) Données moléculaires (AFLP, microsatellites nucléaires, séquences nucléotidiques chloroplastiques).
(2) Données biochimiques basées sur la composition en acides gras du mésocarpe des fruits .
(3) Données écologiques concernant les préférences d'habitat des populations.
(4) Données morphologiques constituées par des mesures des caractères végétatifs et reproductifs.

Toutes ces données concernent l'espèce *Oenocarpus bataua* et ses deux variétés, mais nous avons également inclus autant que possible quelques éléments de comparaison en provenance d'autres espèces du genre *Oenocarpus* et de genres voisins de la tribu *Euterpeae*. L'échantillonnage a été réalisé directement dans les peuplements spontanés de *Oenocarpus bataua* en forêt tropicale. Plusieurs voyages ont été effectués pour la collecte d'échantillons (ADN matériel et fruits) et l'obtention de données (préférences écologiques et mesure morphologiques): février-mars 2004 (Pérou-Equateur); juillet-septembre 2005 (Pérou) et novembre - décembre 2004 (Guyane française).

II

PHYLOGENETIC RELATIONSHIPS AND INTRASPECIFIC VARIATION



Phylogenetic relationships and intraspecific variation of *Oenocarpus bataua* Mart. (Arecaceae)

MANUSCRIT NON SOUMIS

Taxon

Abstract

With the goal to test the evolutionary relationships between *Jessenia* and *Oenocarpus* genera (Arecaceae), a phylogenetic study of the Euterpeae tribu (Arecaceae) was conducted using 3.6 kb of non-coding chloroplastique DNA sequence data. Recovered maximum-parsimony phylogenies support the monophyly of Euterpeae and the inclusion of *Jessenia* as an intraspecific category within *Oenocarpus* genus. The intraspecific variation of *Oenocarpus bataua* (*bataua* and *oligocarpa* subspecies) was analyzed using AFLP data and a polymorphism of inversion. Two populations from Chocó forest and western Amazonia (*bataua*) and one population from Guayana (*oligocarpa*) were analyzed. The AFLP technique generated 69 polymorphic fragments with high frequencies within each population. The higher level of polymorphism was attributed to the variation between populations (54.79%, AMOVA), and a lesser variation within population. Phi-values (Φ) revealed a major genetic divergence between eastern and western populations (western Amazonia *vs.* Guayana = 0.62%; Chocó vs. Guayana = 0.64%). A minor differentiation was found between western Amazonia and Chocó (0.18%). The neighbor joining, PCoA and UPGMA analyses revealed the existence of discrete group of samples that correlates with the geographical origin and taxonomic status. The frequencies of the two loci for the polymorphism of inversion studies were consistent with the former results. Our results pointed out a strong molecular divergence between *bataua* and *oligocarpa.* The taxonomic level of this divergence remains conflictive; *bataua* and *oligocarpa* could represent different species rather than intraspecific categories. This hypothesis was already proposed by Grisenbach & Wendland in 1864.

Keywords: Amazonia, Euterpeae, Molecular phylogenie, *Oenocarpus bataua*, *Jessenia bataua*, genetic differentiation.

Introduction

The palm tribe Euterpeae (Arecaceae) embraces five genera (*Oenocarpus*, *Hyospathe*, *Euterpe*, *Prestoea* and *Neonicholsonia*; Govaerts & Dransfield 2005), and approximately 30 species widely distributed in the Neotropical region. *Oenocarpus* is the second largest genus in the Euterpeae, encompassing about nine species (*O balickii, O. minor, O. mapora, O. distichus, O. circumtextus, O. bacaba, O. bataua, O. simplex, O. makeru*). In particular, *Oenocarpus bataua* Mart. (locally named *ungurahua*, *ungurahui*, *seje*, *mil pesos*) is one of the most conspicuous palms from the tropical forests of northern South America. It is an arborescent (up to 30 m tall), monoiceous, out-crossing palm species highly appreciated by Amerindian tribes and local people for its nutritious fruits, rich in oleaginous compounds (Balick 1986). *Oenocarpus bataua* is a new source of vegetable oils of high quality, worthy of domestication (Balick 1988). However, in spite of its economic potential for the Amazon region, *O. bataua* remains understudied.

Taxonomically, the genus *Oenocarpus* is still confused. The genus *Oenocarpus* with the species *O. bataua* was described in 1823 by Martius in his classic treatment "*Historia Naturalis Palmarum*". Karsten in 1857 described the monospecific related genus *Jessenia* (type *J. polycarpa*). *Jessenia* was separated from *Oenocarpus* on the basis of a larger number of stamens and the ruminate endosperm (Henderson 1995, Henderson *et al*. 1995). *Oenocarpus bataua* was subsequently transferred to *Jessenia* (Burret 1928). A modern taxonomic revision of the *Oenocarpus-Jessenia* complex was carried out by Balick (1986). This work supported the separation of these two genera based on morphological and chemotaxonomical markers. However, Henderson (1999) brought some evidence against the genus *Jessenia* based on morphological and anatomical phylogenetic data that show it been embedded within *Oenocarpus*. Additionally, new botanical findings from the tropical forest in Colombia (Bernal *et al*. 1991, Henderson 1995) documented the existence of a new palm species (*Oenocarpus makeru*) with morphological traits of both *Oenocarpus* and *Jessenia*. Due to these conflicts, phylogenetic relationships between *Jessenia* and *Oenocarpus* remain dubious.

The infraspecific variability of *Oenocarpus bataua* has been little studied over its wide geographical range. Taxonomical monographs like those of Balick (1986) and Henderson (1995) considered two major morphotypes as subspecies or varieties: *oligocarpa* and *bataua*. (1) The *oligocarpa* morphotype is widespread in eastern Amazonia (*i.e*., eastern Venezuela, Guyana, Surinam and French Guiana) and reach Trinidad island. This type

has originally been described as a distinct species named J*essenia oligocarpa* by Grisebach and Wendland in 1864 from a population of Trinidad (Balick 1986). Burret (1928) based on morphological characters proposed the inclusion of *J. oligocarpa* as an infraspecific taxon of *Jessenia bataua*. (2) The morphotype *bataua* is distributed in western Amazonia, from Bolivia to Venezuela, apparently more local in central and eastern Amazonia, with an extension in the Chocó forest of the Pacific coast of Ecuador and Colombia, just reaching Panama, also in the Atlantic lowlands of Colombia and western Venezuela. It also occurs in gallery forest in the *Cerrado* formation of Brazil (Henderson *et al*. 1995, Borchsenius *et al*. 1998) and on Andean slopes up to 1.350 m. elevation. Four species, described according to their geographical distribution and slightly differentiated morphologically (*Oenocarpus/Jessenia weberbaueri*, *O. seje*, *O. polycarpa*, *O. repanda*), have been reduced into synonymy of *Jessenia bataua* subsp. *bataua/Oenocarpus bataua var. bataua* (Balick 1986, Henderson 1995). The exact boundary and pattern of transition between the two morphotypes are still very poorly known.

In this work, we address the following questions (1) Is *Jessenia* distinct from *Oenocarpus*? and particularly is there a molecular evidence that supports the separation of *Jessenia* from *Oenocarpus*?, and (2) to which extent *Oenocarpus bataua* var. *bataua* (or *Jessenia bataua* subsp. *bataua*) is different from *O. bataua* var. *oligocarpa* (or *J. bataua* subsp. *oligocarpa*)? In order to answer the first question, we conducted a cladistic analysis based on non-coding choloplastic DNA (cpDNA). cpDNA has been widely used for phylogenetic inference in the palm family (Baker *et al.* 1999, Asmussen & *Chase* 2001, Hahn 2002). In particular, cpDNA non-coding regions (introns and intergenic spacers) have been applied successfully to resolve relationships among genera and species in various plant groups. At the species level, the phylogenetic utility of cpDNA is more limited (in Baker *et al.* 1999), unless polymorphisms other than substitutions are taken into account.

With respect to the second question, we used a genotyping approach with AFLP markers and a polymorphism of inversion in cpDNA in order to estimate genetic differentiation between the *bataua* and *oligocarpa* morphotypes. Genetic variability of wild palm populations has been studied in a few Neotropical palm species including *Euterpe edulis* (Cardoso *et al*. 2000, Gaiotto *et al*. 2003), *Bactris gasipaes* (Couvrer *et al*. in press), and

Elaeis oleifera (Moretzsohn *et al*. 2002). Molecular markers techniques have been used successfully to evaluate the genetic variability of natural populations (Karp *et al.* 1996, Mueller & Wolfenbarger 1999, Lucchini 2003). Particularly, the capacity of Amplified Fragments Length Polymorphisms (AFLP) as highly variable molecular markers able to resolve small genetic differences and phylogenetic relationships have been demonstrated in several studies (Travis *et al*. 1996, Koopman *et al*. 2001, Zhang *et al*. 2001, Pelser *et al.* 2003). In addition, a polymorphism of inversion detected in one of the cpDNA sequence used for the phylogeny, was employed as a molecular marker to explore the infraspecific variability of *Oenocarpus batau*a. As we propose to test the validity of *Jessenia*, we will use this genus name as distinct from *Oenocarpus*, following Balick (1986) along this study.

Material and methods

Molecular Phylogeny

Eighteen specimens of the tribe Euterpeae were analyzed. *Oenocarpus* was represented by four species, whereas *Jessenia* was represented by three specimens of *J. bataua* subsp. *bataua* and four specimens of *J. bataua* subsp. *oligocarpa*. Two species of each *Euterpe*, *Hyospathe* and *Prestoea* were included in the analyses. The monospecific genus *Neonicholsonia* was represented by one specimen. We used as outgroup eight species from seven other tribes of subfamily Arecoideae, and one species from subfamily Ceroxyloideae (Table 1).

Silicagel-dried material from young leaves was used for DNA extraction. Total genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen). The plastid intergenic spacers *trn*Q-*rps*16, *trn*D-*trn*T, and *psb*C-*trn*fM were amplified and sequenced using the primers sequences listed in Table 2. The *psb*C-*trn*fM spacer was amplified using two pair of overlapping primers (*trn*S-*trnf*M and *psb*C-*trnf*M). PCR amplifications were carried out using FailSafe amplification premix (Epicentre Technologies, Madison, WI) in 25 µl containing, 0.2 µM each primer, 2.5 units of Enzyme mix, 1X PreMix E, and 5 µl of template DNA. The thermal cycling profile was 3 min of initial denaturation at 95°C, followed by 35 cycles of 95°C for 30 s, 50-62°C annealing temperature for 45 s, and 72°C elongation step for 2 min. A final 5 min extension step at 72°C was added. PCR products were sequenced by a commercial laboratory (Genome Express, Grenoble). Contigs of

individual sequences were assembled with Sequence Assembly program (Lasergne software package, DNASTAR Inc., Madison, Wisconsin, USA). Consensus sequences alignment was performed manually using the software PAUP* ver. 4.0b10 (Swofford 2003). Alignment strategy follows the recommendations of Kelchner (2000), four insertions/deletions (indels) and three inversions were additionally coded in a binary matrix.

Maximum Parsimony analysis (MP) was carried out on the noncoding data matrix with the additional binary matrix as described above. This analysis applied a heuristic search strategy with stepwise addition and tree bisection-reconnection algorithms (TBR) as implemented by PAUP. Relative support for each clade was assessed by bootstrap analysis with 1000 resamplings of the data by the heuristic search under unweighted criteria to asses the internal support for clades.

Infraspecific variability (AFLP analysis)

A total of 62 accessions were included in the AFLP analysis, comprising 55 individuals of *Jessenia bataua* and seven individuals representing the genera *Oenocarpus, Euterpe* and *Prestoea* (Appendix 1). Twenty-five individuals of *Jessenia bataua* subsp. *oligocarpa* were collected in French Guiana (Guayana region), 15 individuals of *J. bataua* subsp. *bataua* from Peruvian and Ecuadorian Amazonia (Western Amazonia), and 15 individuals of *J. bataua* subsp. *bataua* from the Pacific lowlands of Ecuador (Chocó; Fig. 1). The distance between individuals sampled in French Guiana and Chocó region was of 1-4 km in average. Western Amazonian individuals were separated by at least 5-150 km.

DNA was extracted from dry leaf tissue using the DNeasy®Plant Maxi and Mini kit (Qiagen Inc., Chatsworth, CA, USA), and following the manufacturer's instructions. Resulting DNA solutions were standardized to a concentration of 50ng/µL**.** Amplified Fragment Length Polymorphisms (AFLPs) allow the detection of polymorphisms of genomic restriction fragments by PCR amplification. *Eco*RI and *Tru*I restriction enzymes were used to digest 10 µL of DNA [50 ng/µL] for each collection. Ligation and preselective amplification were mostly carried out according to the protocol of Vos *et al*. (1995). Selective amplification was performed with three different AFLP primers combinations: *Eco*RI+AAT – *Tru*I+CAT, *Eco*RI+AAT – *TruI*+CAC, *Eco*RI+AGC – *Tru*I+CAT. Selective primer combinations were chosen based on the result of an initial

screening for polymorphism among a limited number of samples. The primers *Eco*RI+AAT and *Eco*RI+AGC were labeled with infrared fluorochromes with waves-

Table 1. List of specimens included in the phylogenie. Taxonomy follows Govaerts & Dransfield (2005).

Sub-family	Tribu	Palm species	Voucher	Group
Arecoideae	*Euterpeae*	*Prestoea acuminata*	H 7650	*Intra group*
		Prestoea shultzeana		
		Euterpe precatorea		
		Euterpe oleracea	JCP 495	
		Hyospathe elegans		
		Hyospathe macrorachis	HB 6421	
		Neonocholsonia watsonii		
		Oenocarpus balickii	JCP 929	
		Oenocarpus mapora	JCP 456	
		Oenocarpus distichus	JCP 496	
		Oenocarpus bacaba	s/n Guy	
		Jessenia bataua sub. bataua (1)	BH	
		Jessenia bataua sub. bataua (2)	JCP 477	
		Jessenia bataua sub. oligocarpa	G18	
	Arecaceae	*Howea belmoreana*	FTG 73337	Sister group
	Arecaceae	*Drymophloeus litigiosus*	FTG 9131A	
	Roystoneae	*Roystonea regia*	FTG 92386	Proxi group
	Manicarieae	*Manicaria saccifera*	H7641	
	Leopoldinieae	*Leopoldinia pulchra*	H7642	
	Geonomateae	*Geonoma oxycarpa*	FTG 86408	
	Cocoseae	*Elaeis oleifera*	FTG 87117	
	Iriarteeae	*Iriartea deltoidea*	H 6340	Out group
Ceroxyloideae		*Phytelephas aequatorialis*	H6247	Out group

Table 2. Chloroplast spacers and inversion primers amplified in this study.

Locus	Primer sequence	Aligned length	T°C anneling
*trn*D-*trn*T (1)	(F) 5'ACC AAT TgA ACT ACAATC CC 3' (R)5' CTA CCA CTg AgT TAA A Ag gg 3'	927 pb	54°C
*trn*Q-*rps*16 (2)	(F)-5' TCg gAg gTT CgA ATC C TT CCg TCC CAg A 3' (R)-5' CAA gTC CgA CgT TgC T TT CTA CCA CAT CgT TT 3'	1232 pb	50°C
*trn*S-*trn*fM (1)	(F)-5' gAg AgA gAg ggA TTC gAA CC 3' (R)-5' CAT AAC CTT gAg gTC Acg gg 3'	~ 1000 pb	62°C
*psb*C- *trn*fM (3)	(F)-5'ATTgTggCATgCggAAgg 3' (R)-5'ggATCggggAAATACCAAATAAgT 3'	~ 800 pb	54.5°C
*psb*C-short- *trn*fM-short (4)	(F)- 5' ATT TGT GGC ATG CGG GAA GG 3' (R)-5' GGA TCG GGG AAA TAC CAA ATA AGT 3'	~ 600 pb	54,5°C

(1) Demesure *et al.* 1995, (2) Hahn 2002, (3) Pintaud (comm. pers.), (4) Chloroplastic Inversion studied

length of 700 nm and 800 nm. Selective amplification products were analyzed on an LI-COR IR^2 automated sequencer.

AFLP-Quantar^{TM}Pro 1.0. (Keygen) was used to score AFLP fragments as present (+), absent (-) or uncertain fragments (?). Only polymorphic fragments discernible in at least 80% of the individuals were used to construct a presence-absence matrix. Neighbor-joining analysis (NJ, Saitou & Nei 1987) and unweighted Pair-Group Method average algorithm (UPGMA, Sneath & Sokal 1973) were performed to visualize relatedness between individuals. Pairwise genetic distances for all individuals (including outgroup) were calculated from the presence-absence matrix using: (i) Nei & Li index (1979), and (ii) mean character distance. The tree was bootstrapped using 1.000 replicates. Neighbor-Joining analysis and UPGMA were conducted with PAUP* 4.0b10 (Swofford 2003).

In addition, a Principal-coordinate Analyses (PCoA) was carried out on a pairwise similarities matrix between individuals using the Jaccard and the Simple Matching coefficients. Before PCoA analysis, both coefficients were converted to distances using the formula D=1-S. PCoA was performed for two set of data: (*i*) *oligocarpa* (n=25) plus *bataua* (n=29, from western Amazon and Chocó), and (*ii*) only *bataua* subspecies (n=29). PCoA and matrix construction were performed with R-package version 4.0d6 (Casgrain & Legendre 2001).

An analysis of molecular variance (AMOVA, Excoffier *et al*. 1992) was performed to assess genetic variability among AFLP genotypes considering three populations of South America: Guayana (subsp. *oligocarpa*), western Amazon (subsp. *bataua*) and Chocó (subsp. *bataua*). In order to homogenize the number of individuals per each region, we randomly selected 14 individuals from Guayana and western Amazonia. For AMOVA analysis, overall genetic variation was divided into intrapopulational and interpopulational components. Genetic differentiation (Φ-statistics) among pairs of *Jessenia bataua* populations and their levels of significance were calculated from the AMOVA analysis. The Φ-statistics is analogous to traditional F_{ST} statistics (Fixation Index).

Figure 1. Area of distribution of Jessenia bataua subsp. bataua and subsp. oligocarpa in northern South America and sampling areas.

AMOVA analysis and population comparisons (Φ-statistics) were computed with a matrix of squared-distances among all AFLP haplotypes using the coefficient *haploid pairwise differences*; which is expressed by the formula $d_{xy}=\Sigma\delta_{xy}$ (ι), where δ_{xy} (ι) is the Kronecker function, equal to 1 if the alleles of the *i*-th locus are identical for both haplotypes, and equal to 0 otherwise. The significance of the variance components and Φ-statistics were tested using a permutation test with 1.000 replicates. AMOVA analysis and population comparisons were performed using ARLEQUIN version 2.0 software (Schneider *et al*. 2000). The individual #14 from Chocó region was excluded from our analysis since this specimen showed an uncommon pattern of AFLP bands.

Polymorphisms of inversion

A genetic inversion was used as molecular marker to test the genetic differentiation between subspecies *oligocarpa* and *bataua*. This minute inversion as short as 7 base positions (bp) in lenght and bordered by a nearly perfect ~12 bp inverted repeat was detected in the plastid intergenic region *psb*C-*trn*fM in various palm species. Primers definition for this inversion was performed using the software Primer Select of Lasergene package, Version 5.5 (DNASTAR, Inc. USA). Location and additional information of this inversion is given in the table 2.

Ten individuals for each subspecies were amplified with these primers and subsequently sequenced. Contigs of individual sequences and fragments were assembled using the software SeqmanII of Lasergene package, Version 5.5 (DNASTAR, Inc. USA). PCR products were sequenced by Genome Express (Grenoble).

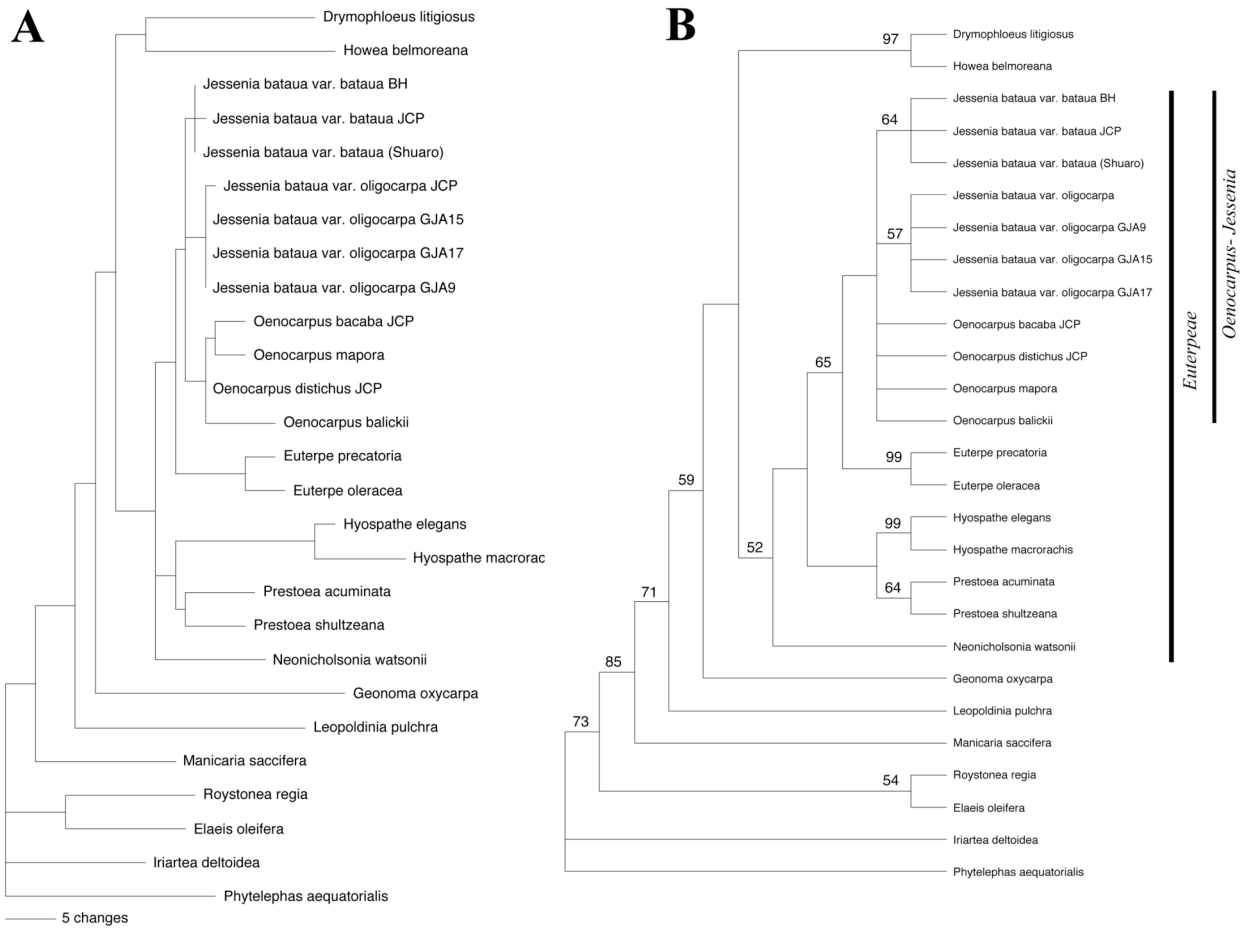


Figure 2. (A) One tree chosen from 26 equally parsimonious trees resulting from analysis of cpDNA in Euterpeae tribe. (B) Strict consensus of 26 MP trees for noncoding regions of cpDNA (trnD-trnT, trnQ-rps16, psbC- trnfM). Bootstrap values > 50% are given above the lines.

Results

Parsimony analysis with cpDNA

The *trn*D-*trn*T spacer included 927 bp with 17 (1.8%) characters phylogenetically informative. Two indels of 2 bp and 30 bp were found in this spacer. The first one was observed for two species of Oenocarpus (*O. distichus*, *O. mapora*), and the second indel was restricted to *Oenocarpus-Jessenia-Euterpe* clade. In addition, an minute inversion of 6 bp in lenght was present in several species. The *trn*Q-*rps*16 spacer comprised 1.232 bp, 19 characters (1.5%) were parsimony informative. Only one minute inversion of 2 bp was detected which was synapomorphic to Euterpea tribu. The *psb*C-*trn*fM spacer was 1.464 bp long with 13 characters (0.88%) phylogenetically informative. Two minute inversions (7bp and 3bp) were identified from this region. In addition, an indel of 6 bp was coded for one species of Euterpeae (*Euterpe precatorea*) and one of the proxy group (*Geonoma oxycarpa*).

A final matrix of 3630 characters, with only a 1,5% of characters phylogenetically informative was analyzed. Parsimony analysis of the non-coding sequences data recovered 26 most-parsimonious trees. These trees were 252 steps long, with a consistency index of 0.60 (excluded uninformative characters), and a retention index of 0.75. A dendrogram tree and the strict consensus tree are shown in the Fig. 2. with bootstrap values.

The monophyly of tribe Euterpeae is established but with moderate boostrap support (52%). *Hyospathe*, *Prestoea* and *Euterpe* were also resolved as monophyletic, with high bootstrap values > 60%. *Jessenia* is embedded within *Oenocarpus* and the *Oenocarpus*/*Jessenia* clade is supported by a bootstrap value of 52%. Another clade was the monospecific genus *Neonicholsonia*. Intergeneric relationships remained unresolved.

The specimens of *Oenocarpus* and *Jessenia* included in the phylogenetic analysis formed a polytomy. The specimens of *Jessenia bataua* subsp. *bataua* formed a monophyletic group with 64% of bootstrap, the specimens of *Jessenia bataua* subsp. *oligocarpa* also grouped together with 57% of bootstrap, but these two subspecies did not form a clade.

AFLP analysis

Amplifications with three AFLP primers generated a total of 69 polymorphic bands across the entire data set. The number of bands for the primers AAT-CAT, AAT-CAC, AGC-CAT was 39, 19 and 11, respectively. Within each population, the majority of bands occurred at high frequencies. The partition of the variance as revealed with AMOVA analysis for the AFLP genotypes matrix showed that 54.79 % (P<0.001) of the variation was explained by the differences among populations (Guayana, western Amazon, Chocó); the rest (45.21 %; P<0.001) was attributed to the variation within populations (Table 3).

Comparisons of pairs of population samples (Φ-statistics) revealed a strong genetic differentiation between *oligocarpa* (Guayana) and *bataua* populations (western Amazon, Chocó). Φstatistics pointed out a major differences between *bataua* (Chocó) *vs. oligocarpa* (Φ=0.64, P<0.001) followed by *bataua* (western Amazon) *vs. oligocarpa* (Φ=0.62, P<0.001). A minor but significant differentiation was detected between *bataua* individuals from western Amazon and Chocó (Φ=0.18, P<0.001).

Table 3. Analysis of molecular variance (AMOVA). Distribution of the variance among populations (Guayanas, Western Amazonia and Chocó) and within populations. Variances components were computed by AMOVA for a matrix of AFLP genotypes along three population with 14 individuals each one. *P*-values were estimated by permutational analysis of the null distribution of the variance components using 1.000 permutations. Abbreviations: d.f, degrees of freedom; SS, sum of squares; ***P***-values, the significance of the variance components (*** = P<0.001).

Source of variation	d.f.	SS	Variance components	Percentage of variation	P-*values*
Among populations	2	219.19	7.39	54.79	***
Within population	39	237.92	6.10	45.21	***

The NJ tree and UPGMA analyses based on Nei & Li index and mean character distance showed that genotypes of *Jessenia bataua* individuals were strongly grouped in function

of their geographical origin or morphotype. The figure 3a summarizes the clustering patterns among the methods and indices applied. A striking feature of these analyses was the grouping of all *Jessenia/Oenocarpus* species into a single cluster with a bootstrap value > 50%. Inside of this cluster, all individuals of *J. bataua* were grouped into two major sub-clusters: (i) individuals from Guayana or *oligocarpa* subspecies (bootstrap value > 50%), and (ii) individuals from western south America or bataua subspecies (western Amazon plus Chocó, bootstrap value > 50%). Other species inside *Oenocarpus* (*O. bacaba*, *O. distichus*, *O. mapora*) were placed indistinctly in either of the former sub-clusters. Inside of these sub-clusters, there was not an evident genetic structure. Minor differences in the topological rearrangement in out-group species were observed among the different analyses. The NJ analysis based on mean character distance showed the most natural pattern of grouping with the highest bootstrap values (Fig. 3b). The NJ and UPGMA analysis revealed a genetic differentiation among *oligocarpa* and *bataua* populations.

The results of PCoA were in agreement with those from NJ and UPGMA analyses. PCoA suggested an association between the genetic differentiation and the geographical origin. *Oligocarpa* and *bataua* morphotypes were clearly separated in the multivariate space of the PCoA (Fig.4a, b). The first two eigenvectors accounted for 44.32% and 35.60% of the variance explained for PCoA analysis using Jaccard and Simple matching coefficient, respectively. In addition, PCoA-plot among *bataua* individuals (western Amazon and Chocó; Fig. 4c, d) showed a continuous gradient in the molecular diversity. No evident pattern was detected inside Guayanan and Western Amazonian individuals. The first two eigenvectors accounted 27.16% and 25.45% of the variance explained for PCoA analysis using Jaccard and Simple matching coefficients, respectively.

Polymorphism of inversion

A screening using ten individuals of each subspecies (*oligocarpa* and *bataua*) displayed results consistent with those of AFLP. Individuals from each subspecies had a different state of the inversion except for one *oligocarpa* individual which had the state for which *bataua* is monomorphic (Fig. 5).

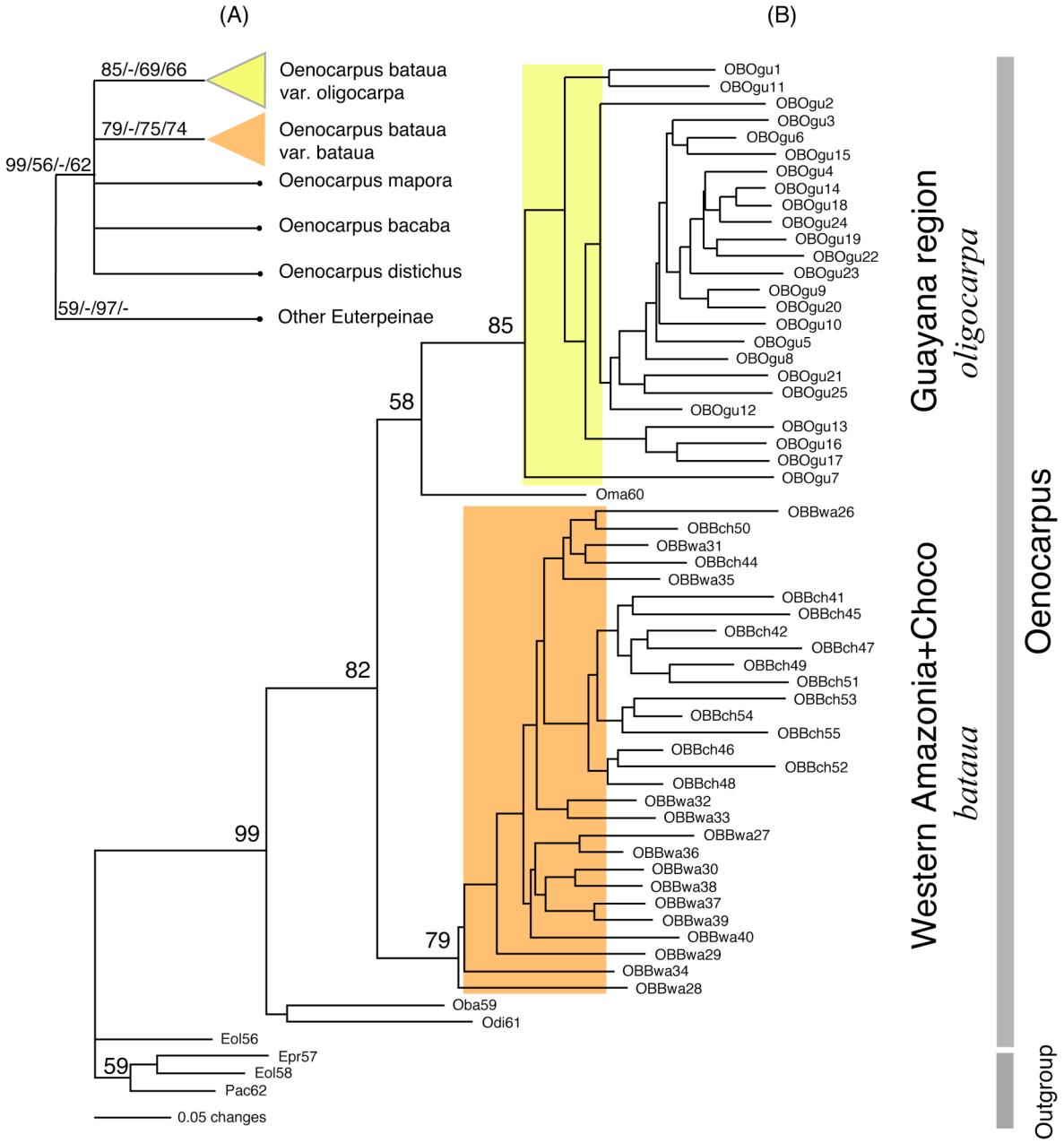


Figure 3. (a) Synthetic tree summarizing the results of four trees using UPGMA and Neighbor-joining (NJ) analysis. Common branches for all trees are represented with their bootstrap values in the following order: UPGMA with Nei & Li index/NJ with Nei & Li index/UPGMA with mean character distance/NJ with mean character distance. b) Neighbor-joining dendrogram based on mean character distance among 55 individuals of Jessenia bataua and seven outgroupe species. Only Bootstrap values > 50 % are showed above branches. Bootstrap values were computed by 1.000 permutations.

Discussion

Molecular Phylogeny: The non-coding cpDNA sequences included in this study revealed low levels of sequence divergence for Euterpeae palms (1.5 % of character phylogenetically informative). This fact has already been reported in the literature for a wide range of palm species (Wilson *et al*. 1990, Baker *et al.* 1999). However, despite the limited number of informative sites, the low levels of conflict among the data help to resolve the monophyly of Euterpeae and of its genera.

The resulting phylogeny from cpDNA corroborated the monophyly of the tribe Euterpeae as reported by Henderson using morphological characters (1999). At the genus level, five clades corresponding to each Euterpeae genus (*Hyospathe, Euterpe, Prestoea, Neonicholsonia, Oenocarpus-Jessenia*) were obtained, but their phylogenetic relationships remained unresolved. Most noteworthy is the inclusion within a single clade of all species of *Oenocarpus* and *Jessenia*. In particular, there is no synapomorphy characterizing either *Jessenia* or *Oenocarpus sensu stricto* in the cpDNA sequences. Therefore, there is no molecular support for the separation of *Jessenia* from *Oenocarpus* as it was accepted by Balick (1986).

At the subspecific level, the strict consensus tree (Fig. 2b) grouped *bataua* and *oligocarpa* specimens in two separate clades. The two clades differed by four substitutions in *TrnD-TrnT* and *TrnQ-rps16* spacers and also by a 7 bp inversion. These results reveal a strong molecular divergence between the two subspecies. This molecular divergence correlates with the morphological differentiation pointed out by Grisebach and Wendland in 1864 (see Balick 1986). These authors considered both taxa (*bataua* and *oligocarpa*) as two distinct species. The hypothesis that these two taxa might indeed represent two different species need to be tested with further molecular and reproductive data.

Utility of AFLPs and polymorphism of inversion: AFLPs and polymorphism of inversion were suitable markers for exploring the molecular variation among Oenocarpus bataua populations. Both source of molecular information revealed a strong molecular differentiation between bataua and oligocarpa subspecies. However, the polymorphism of inversion requires a previous knowledge on genome sequence, which is a technical disadvantage for species poorly known. In other hand, AFLP technique does not require

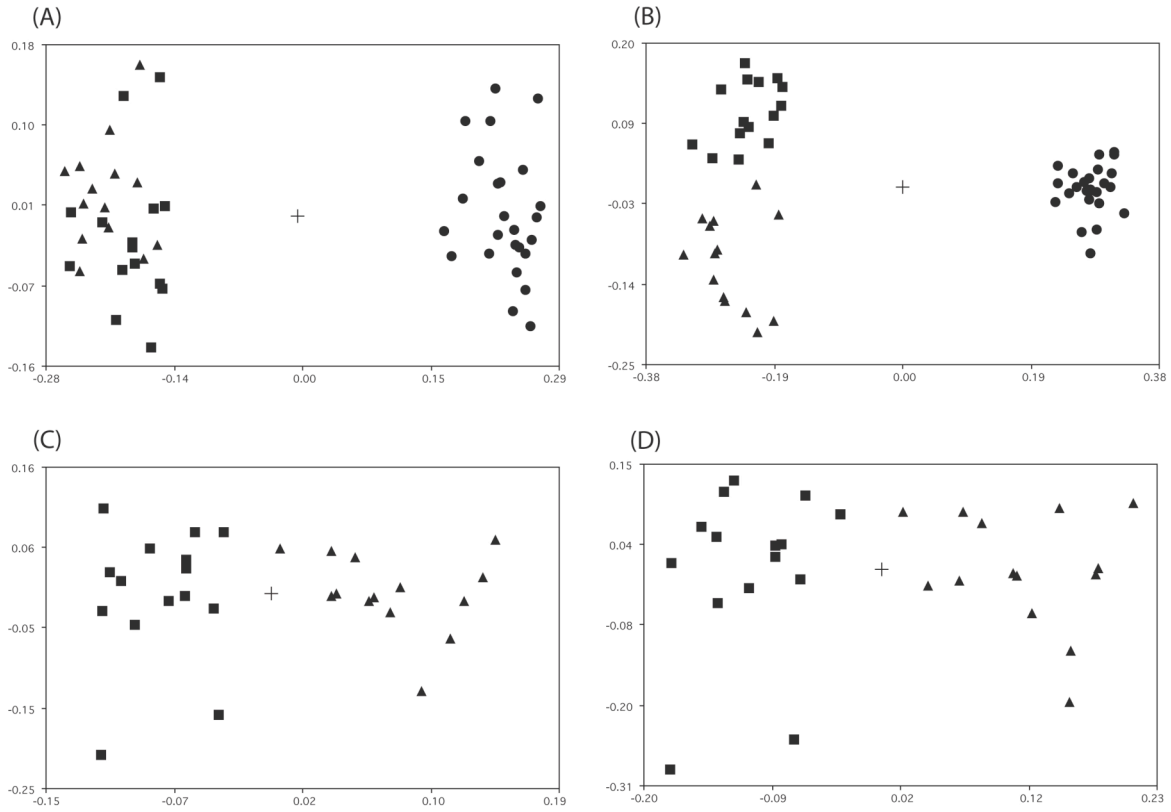


Figure 4. Two-dimensional plots of the principal coordinates (PCoA) of Jessenia bataua individuals:(a) PCoA-plot of all 55 individuals based on simple matching coefficient, (b) PCoA-plot of 55 individuals based on Jaccard coefficient, (c) PCoA-plot of 29 individuals based on simple matching coefficient, and (d) PCoA-plot of 29 individuals based on Jaccard coefficient.

Symbols used

triangles = individuals of Jessenia bataua subsp. bataua from western Amazonia

squares = individuals of Jessenia bataua subsp. bataua from Chocó forest

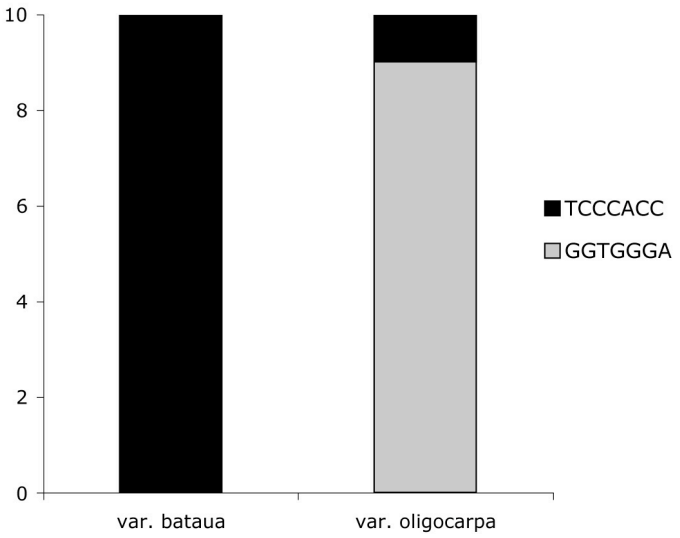
circles = individuals of Jessenia bataua subsp. oligocarpa from Guayana region

initial sequence information and it produces a high number of polymorphic markers that account for the accuracy of this molecular tool.

Infraspecific variability of Jessenia bataua: The genetic variation observed in wild populations of *Jessenia bataua* is slightly higher among populations (54.79%, AMOVA) than within populations (45.21%). Compared with studies performed with natural populations of tropical trees (Muluvi *et al*. 1999, Cardoso *et al*. 2000, Cavers *et al*. 2003), our analyses showed a fairly higher value for the variation among populations. The highest level of genetic differentiation (Φ value) was found between *bataua* and *oligocarpa* subspecies; and to a lesser extent within *bataua* populations. Therefore AFLP results also point out to a strong differentiation between *oligocarpa* and *bataua* taxa. As an out-crossed wild species, the level of variation within *J. bataua* populations was also high (Aide *et al*. 1998, Cardoso *et al*. 2000, Sensi *et al*. 2003). However, it has to be taken into account that our populational samplings were realized over relatively wide geographical ranges. In particular, the spatial distance between the northernmost and southernmost individual for the Western Amazonian population was *c.a.* 500 km.

The genetic differentiation between *bataua* and *oligocarpa* observed resulted from substantial differences in allele frequencies among populations. This might be explained in different ways. The *bataua* populations from Western Amazon/Chocó and *oligocarpa* from Guayanas correspond to the geographical extremes of the distribution range of the species in South America. Therefore, a differentiation by distance can be invoked, which could be transformed in a continuum with a more homogeneous geographical sampling. However, the hypothesis of a genetic structure based only on distance is not well supported by the botanical evidence. Botanical inventories in Amazonia have reported *bataua* populations in areas fairly close to the Guayana like Central or even Eastern Amazonia (Henderson 1995).

Another explanation would be the effect of the geological history of the Amazon on the evolution of the species, which resulted in two clearly differentiated allopatric or parapatric taxa. Ancient populations of *Jessenia bataua* from western Amazonia and Guayana might have been physically isolated from the late Tertiary and Quaternary by several geological events like marine transgression, climatic changes, continental flooding



var. bataua **TTCTGGCTCGGCTATCCCACCTAGCCGAGCCA**

var. oligocarpa **TTCTGGCTCGGCTAGGTGGGATAGCCGAGCCA**

Figure 5. Polymorphism of inversion. Frequencies of alleles found for 20 individuals (10 for each subspecies) of *Jessenia bataua.*

(Prance 1982,, Frailey *et al.* 1988, Haffer 1997, Hooghiemstra & van der Hammen 1998, Nores 1999, Colinvaux *et al.* 2000). These geological events might have restricted the pollen and seed movements between western Amazonian and Guayanan populations. This kind of biological divergence has largely been documented by the distribution patterns of various sister taxa, which show disjunctions between western Amazonia and Guayanas (Mori 1991, Berry *et al.* 1995, Renske 1997).

Although the molecular divergence among *bataua* populations (Chocó *vs.* western Amazonia) was low (Φ-statistics= 0.18), compared with the differentiation between *bataua* and *oligocarpa* taxa, it was statistically significant. The figure 4c and d shows that individuals from Chocó and western Amazonian are not mixed with each other, they rather form a continuous gradient of molecular variation. This genetic pattern suggests that in spite of the presence of a massive physical barrier (the Andes), multiple genetic flux have probably occurred between Amazonia and Chocó, through Andean valleys. Several modern plant genera were already present in the lowlands of South America about 60 millions years before present (Ma BP, Hooghiestra *et al*. 2002). The upheaval of northern Andes (16-10 Ma BP; Hooghiestra *et al*. 2002) split the lowlands of northern South America into the actual Chocó and Amazonia regions. However, several dispersal corridors through Andean valleys as the Huancabamba Deflection (northern Peru-southern Ecuador, Simpson 1975) or the Caribbean lowlands around the northern end of the Andes (Brumfield & Capparella 1996) favored a continuous genetic flux between these regions particularly during the warmer periods. Even today, in southern Ecuador, Chocó and Amazonian populations of *Jessenia bataua* reach the Andean slopes until 1.00-1300 m of altitude reducing the isolation area between Chocó and Amazonian populations and increasing the likelihood of genetic flux via disperses.

Inversion: Small inversions bordered by inverted repeats are common features of chloroplast genome evolution (Graham & Olmstead 2000). We reported and characterized a chloroplast inversion discovered in the course of a DNA sequencing survey into Euterpeae tribe. This small inversion (7bp) was successfully used as molecular marker to differentiate infraspecific categories in *Jessenia bataua*.

The minute inversions have been reported not to be optimal phylogenetic markers (Kelchner & Wendel 1996, Graham *et al*. 2000). Small inversions seem to be highly

susceptible to parallelism and reversal (Graham *et al*. 2000, Graham & Olmstead 2000) and consequently they convey higher rates of homoplasy (Graham *et al*. 2000). This behavior was observed within our dataset when the two states of this inversion were distributed indistinctly among Euterpeae species and the outgroups.

The most remarkable feature of this minute inversion was the consistent segregation of its states between *bataua* and *oligocarpa* subspecies. A single individual from *oligocarpa* subspecies showed the state corresponding to *bataua*, while it was clearly included in the *oligocarpa* cluster resulting from AFLP data. The presence of this atypical *oligocarpa* individual could be explained by (i) the high homoplasy attributed to inversions, or (ii) by an hybrid interspecific between *Oenocarpus bacaba* x *J. bataua* subspecies *oligocarpa*. Our sequence data shows that *O. bacaba* shares the same state of inversion corresponding to *bataua* subspecies. The hybrids between these palm species in Guayana region have been reported in the literature (Henderson 1995).

Taxonomical status of **Jessenia bataua:** It is now clear that the genus *Jessenia* should be considered as a synonym of *Oenocarpus*, based on the unambiguous molecular phylogenetic data. Within the species, the most recent taxonomical monographs (Balick 1986, Henderson 1995) interpreted the morphological differences between western and eastern populations as the evidence of two distinct varieties or subspecies. However, our findings suggest a greater differentiation between *bataua* and *oligocarpa* taxa which have been previously considered as separate species (Wessels Boer 1965, 1971; Grisebach & Wendland 1864 in Balick 1986). In particular, we have found no evidence of sister relationships between *bataua* and *oligocarpa* taxa in any of the analyses conducted, but the results remain inconclusive because interspecific relationships within *Oenocarpus* are not resolved.

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Appendix 1. List of *Jessenia bataua* collections.

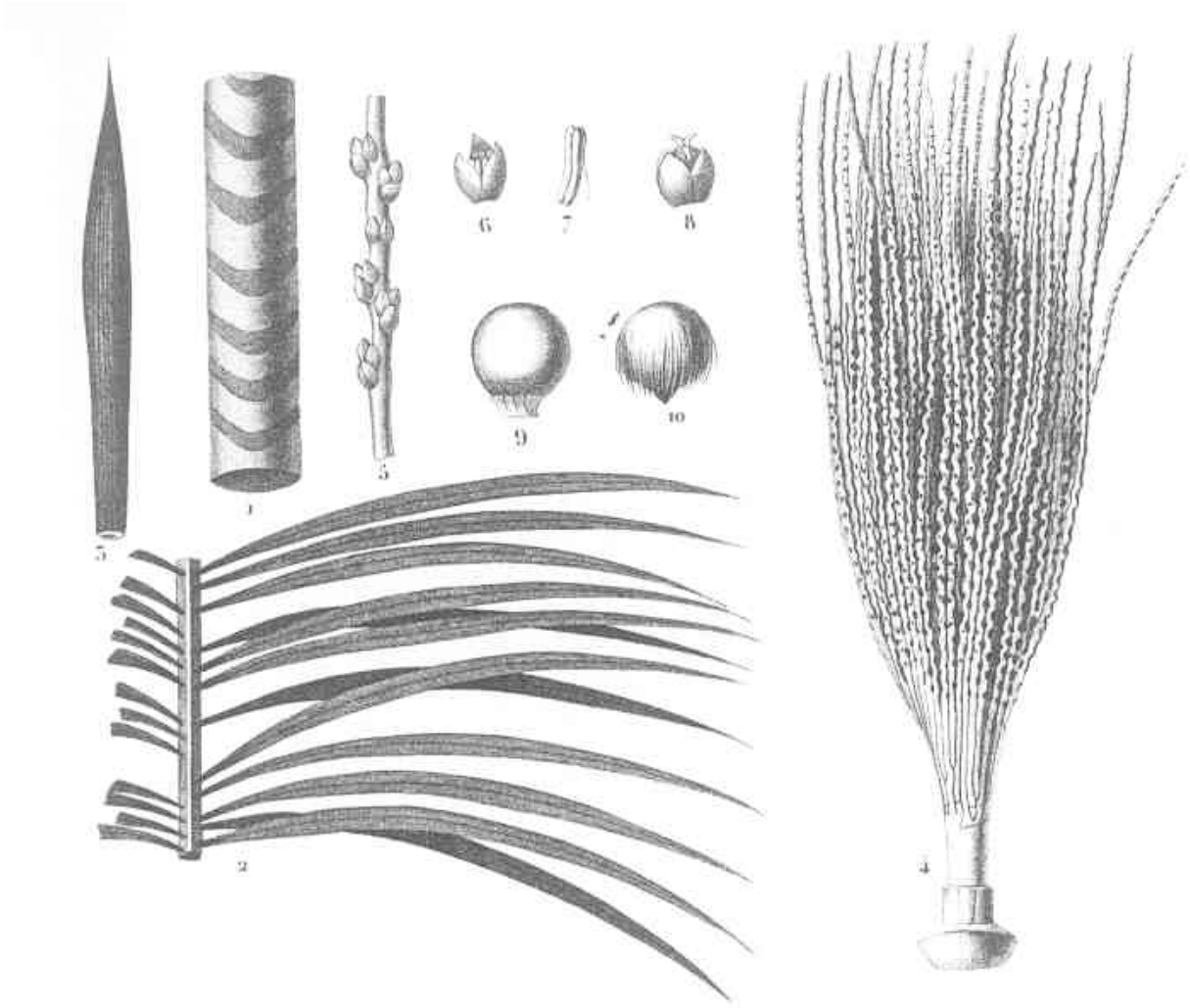
Collections	Taxa	Region	Voucher	Country
OBOgu1 — OBOgu25	*Jessenia bataua var. oligocarpa*	Guayana	Perez 783 (CAY)	French Guyana
OBBwa26 — OBBwa30	*Jessenia bataua var. bataua*	Western Amazonia	FK 4368 (USM)	Peru
OBBwa31 — OBBwa40	*Jessenia bataua var. bataua*	Western Amazonia	RM 97 090, RM 97 059 (QCA)	Ecuador
OBBch41 —OBBch55*	*Jessenia bataua var. bataua*	Chocó	Balslev 62005 (QCA)	Ecuador
Eol56	*Euterpe oleracea*	Central Amazonia	Noblick 5023 (FTG)	Brazil
Epr57	*Euterpe precatorea*	Western Amazonia		Peru
Eol58	*Euterpe oleracea*	Chocó		Ecuador
Oba59	*Oenocarpus bacaba*	Guayana	Perez 850 (CAY)	French Guyana
Oma60	*Oenocarpus mapora*	Chocó	Borchsenius 191 (QCA)	Ecuador
Odi61	*Oenocarpus distichus*	Southern Amazonia	FTG 88579	Bolivia
Pac62	*Prestoea acuminata*	Chocó	Jaramillo & Sak 5940 (QCA)	Ecuador

* OBBch43 was excluded from the analysis.

III

STRUCTURE INFRASPÉCIFIQUE AUX NIVEAUX BIOCHIMIQUE ET MORPHOLOGIQUE

(statut des variétés)



III-*a* Variation in mesocarp fatty acid composition of *Oenocarpus bataua* Mart. *var. bataua* and *var. oligocarpa* (Arecaceae)

MANUSCRIT EN PREPARATION
Biochemical Systematics and Ecology

Abstract

The fatty acid composition of mesocarp oil from fruits of 38 wild individual palm trees of *Oenocarpus bataua* growing in different localities of Western Amazonia (*bataua* variety) and French Guiana (*oligocarpa* variety) was analyzed by gas chromatography. Thirty-one and 16 fatty acids were detected in *bataua* and *oligocarpa* varieties, respectively. Fifteen fatty acids occurring in both varieties plus lipid content were used to perform a multivariate analysis. Principal Component Analysis (PCA) revealed a clear-cut distinction between *bataua* and *oligocarpa* varieties using mesocarp oil fatty acid composition and lipid content. Twelve of the fifteen fatty acids analyzed showed significant differences between varieties. The *oligocarpa* variety was characterized by a higher concentration of lipid content, and palmitic, cis-vaccenic, alpha-linolenic, myristic and lauric acids; while *bataua* variety contained higher concentrations of oleic, stearic, linoleic, palmitoleic, margaric and 15:0 acids. The biochemical divergence among *O. bataua* varieties is in perfect agreement with the botanical evidence.

Keywords: *Oenocarpus bataua*, *oligocarpa* variety, *bataua* variety, Amazonia, fatty acids

Introduction

The Amazon forest harbors one of the highest plant biodiversity of the planet (Vásquez-Martínez, 1997) and the numerous species producing oily fruits and seeds represent a major economic and nutritional potential for this region. In particular, the fruits of palm species (Arecaceae) have been traditionnally used by Amerindians and householders as source of oils and waxes (Lleras & Coradin, 1988; UNDP, 1992; Borgtoft Pedersen & Balslev, 1993). In this region, more than one hundred fifty palm species have been recorded (Henderson 1995), some of them with a real potential as new sources of vegetal oil.

Oenocarpus bataua Martius is a wild palm species with a great potential for domestication due to the oleaginous content of its fruits (Balick, 1981; 1988; Johnson, 1996). This palm species is widely distributed in the northern part of South America, reaching Panama and Trinidad (Henderson, 1995). Two major morphological types or varieties have been recognized at the infraspecific level: *var. bataua* and *var. oligocarpa* (Balick, 1986; Henderson, 1995). The *bataua* variety is mainly distributed in the Amazon valley, with an extension in the Choco forest of the Pacific coast of Ecuador and Colombia. The *oligocarpa* variety is widespread in eastern Amazonia (i.e., Venezuelan Guayana, Guyana, Surinam and French Guiana) and Trinidad. Morphological traits like the presence of trichomes or a waxy covering on abaxial surface of the pinnae, shape of rachillae, number and position of the pistillate flowers on the rachillae, and size of the staminate flowers have been used to distinguish the varieties (Balick, 1986).

It has largely been demonstrated that the fatty acid composition can serve as taxonomical markers (Mongrand et al., 2001; Mongrand et al., 2005; Mayworm & Salatino, 2002; Goren et al., 2006). The sole reference for palm trees comes from Opute (1978). This work evidenced that seed lipids are good markers to explore taxonomic relationships in the palm family.

The diversity of fatty acids has been poorly inventoried in Neotropical palms. For *Oenocarpus bataua*, several studies describing the chemical composition of the fatty acids of the mesocarp have been published (Balick & Gershoff, 1981; Lleras & Coradin, 1988; Lubrano & Robin, 1997; Aleman et al., 2002), but none addressed the intraspecific variability of fatty acids, which is the focus of the present study.

Materials and methods

Specimen collection

The fruits of *Oenocarpus bataua* var. *bataua* were collected from wild specimens occurring in lowland forest close to the locality of Jenaro Herrera (Peru, Western Amazonia); while the fruits of *O. bataua* var. *oligocarpa* were sampled close to the locality of Regina, Approuague basin (French Guiana, Guayana region). Ripe fruits (dark purple and soft to the touch) from 16 and 22 individuals were collected in Western

Amazonia and Guayana region, respectively. The geographical distance among individuals varied between 100 m to 10 km in French Guiana and 500 m to 12 km in Peru. For each individual about 10-15 fruits were collected randomly directly from the infructescence, and kept into plastic bag with abundant silica gel. Voucher specimens were deposited at the Herbarium of Cayenne (CAY) in French Guiana and at the Herbarium of Universidad Mayor San Marcos (USM) in Peru. Fieldwork was carried out during the months of February/March 2004 in Peru and November/December 2005 in French Guiana.

Samples extraction and gas chromatography analyses

For each individual, the mesocarp was isolated mechanically from 5-8 fruits. The mesocarp was reduced into a fine powder using an analytical grinder (IKA A10, IKA® Germany). The powder obtained was divided into three samples of 300 mg per individual (replicates) for fatty acid composition analyses, and of 200 mg for water content measurement. The water content of samples was estimated by desiccation of powders in an oven at 105°C overnight.

Total lipid content was extracted using a modified Folch method (Folch et al., 1957). Lipid content was determined gravimetrically after complete solvent evaporation under a nitrogen stream at 40°C and expressed on a dry weight basis. Fatty acid methyl esters (FAMEs) were prepared according to the NORME NF ENISO-5509 method (AFNOR, 2000). FAMEs were directly injected in GC (HP 5890 system with flame ionisation detection and a Famewax capillary column (RESTEK, 30m x 0.25mm x 0.25µ). The analyses were carried out in program temperature mode from 185°C to 225°C at 4°C/min and then in the isothermal mode for 10 min at 225°C**.** FAMEs were identified by comparison with known standards (Sigma) and were quantified as a percent of total fatty acids (w/w). For each individual, the fatty acid composition was analyzed in triplicate (from three different lipid extract).

Statistical analyses

A multivariate approach was performed to explore the biochemical structure of *Oenocarpus bataua* samples using fatty acids plus lipid content. The statistical analysis

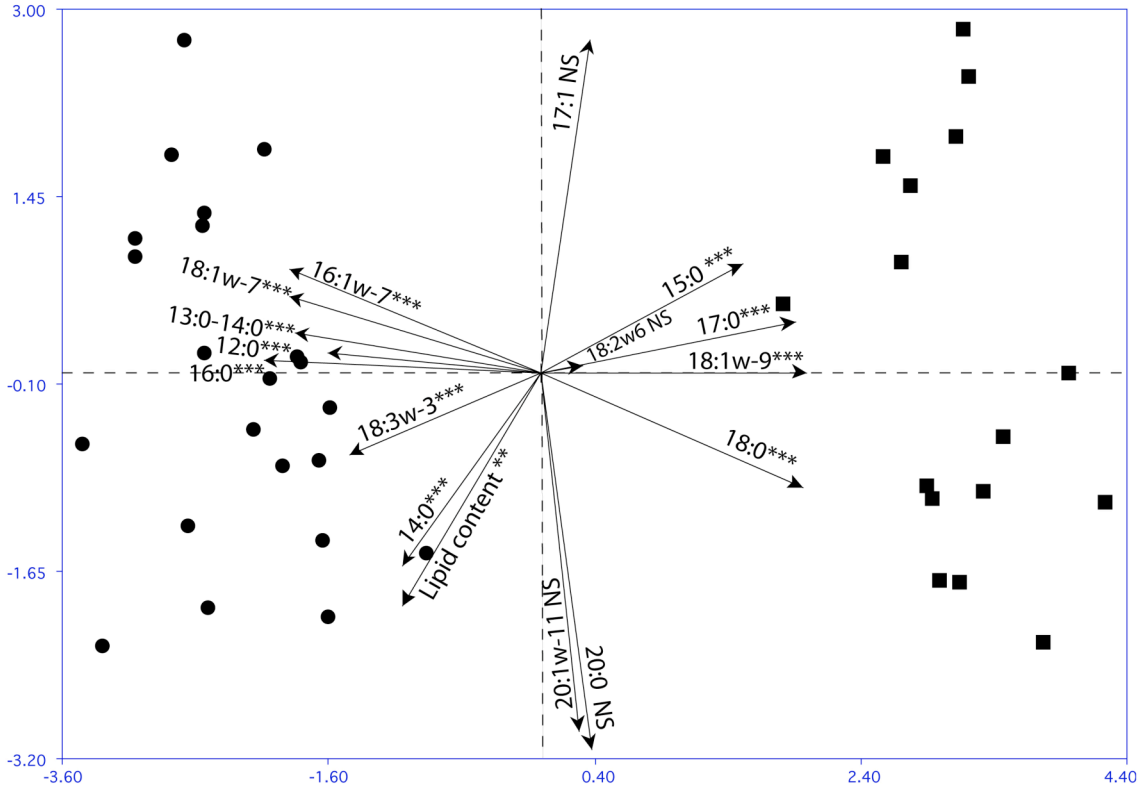


Figure 1. Relative position of the 38 samples fruits of *Oenocarpus bataua* in a space defined by two principal components (PCA). Principal components were constructed with the information of 15 fatty acids plus lipid content. Solid circles represent samples from Guayanas region (*oligocarpa* variety), and solid squares correspond to Western Amazonian samples (*bataua* variety). Arrows represent the eigenvector loading on the first two axis. For a graphical purpose, these former values were multiplied for a constant. Statistical differences of 15 fatty acids plus lipid content between *bataua* and *oligocarpa* samples were evaluated by the Wilcoxon test: *** P<0.001, NS P>0.05

was carried out using the percentage of each fatty acid over total fatty acids. The values for each replicate (n=3) were averaged and used to perform numerical analyses. For statistical purpose, fatty acids found in only one variety of *O. bataua* or with mean values ≤ 0.01 % were excluded from the analysis. A final set of 15 fatty acids plus lipid content was used as variables for the statistical analysis. A Principal Component Analysis (PCA) was computed from the correlation matrix using a set of 15 fatty acids plus the lipid content as descriptors (p = 16); and individuals as objects (n = 38 individuals). Before PCA, a multinormality test using the test of Dagnelie as implemented in R-Package (Legendre & Legendre, 1998) was performed. All statistical analyses were carried out using the software R-package (Legendre & Legendre 1998). The effect of each variable (p = 16) on population variation was evaluated by the Wilcoxon test using the software JMP Version (3.1.5).

Results

Thirty-two fatty acids were detected from the oil of *Oenocarpus bataua* fruits (Table 1, 2). Fifteen fatty acids were found in both varieties (*bataua* and *oligocarpa*), sixteen fatty acids were only found in *bataua* variety, and a single fatty acid was restricted to *oligocarpa* variety. Both varieties had similar qualitative composition at least for the major fatty acids (Table 1) with oleic and palmitic acids being the most abundant fatty acids, followed by cis-vaccenic, stearic, linoleic and alpha-linolenic acids.

Quantitatively, the fatty acid composition of the mesocarp of *Oenocarpus bataua* fruits varied significantly among varieties. Except linoleic, arachidic, gondoic and 17:1 acids, all fatty acids plus lipid content showed significant differences (Wilcoxon test, p<0.05) between *bataua* and *oligocarpa* varieties (Figure 1).

The results of PCA are presented in table 3 and figure 1. The hypothesis of multinormality was accepted by the test of Dagnelie with a significance level for *Ho* of 0.01. The first three components were informative (λ_K larger than the mean of the λ's). A high percentage of the total variation was explained by the first component (48%). The most discriminant fatty acids along component 1 were: palmitic, oleic, stearic and margaric acids. Samples of *Oenocarpus bataua* corresponding to *bataua* and *oligocarpa* varieties were clearly separated on this axis. The second component explained 14% of the total

variation, and it was mainly characterized by arachidic, gondoic and 17:1 acids. The third component represented 10% of the variation with linoleic acid being its most important variable.

Table 1. Overall mesocarp fatty acids composition and lipid content of *Oenocarpus bataua*. Data shown are the mean values of 16 and 22 fruit samples from Western Amazonia and Guayana region, respectively. Values are expresed as % fatty acid over total fatty acids.

			Western Amazonian *bataua* variety *n*=16 ind.				Guayana region *oligocarpa* variety *n*=22 ind.			
#	Shorthand designation	Trivial Name	Mean	±SD	Max	Min	Mean	±SD	Max	Min
1	12:0	Lauric	0.004	0.004	0.01	0	0.013	0.009	0.037	0
2	13:0-14:0	Undetermined	0.031	0.032	0.107	0	0.148	0.036	0.195	0.085
3	14:0	Myristic	0.069	0.017	0.108	0.036	0.112	0.059	0.294	0.051
4	15:0	-	0.353	0.071	0.5	0.264	0.222	0.048	0.366	0.149
5	16:0	Palmitic	11.966	1.200	14.069	9.684	22.608	1.999	25.96	19.989
6	16:1	Palmitoleic	0.139	0.027	0.19	0.096	0.081	0.016	0.118	0.066
7	17:0	Margaric	0.076	0.009	0.097	0.066	0.050	0.006	0.064	0.037
8	17:1	-	0.074	0.017	0.113	0.054	0.067	0.010	0.09	0.051
9	18:0	Stearico	2.570	0.534	3.503	1.3	1.153	0.130	1.412	0.875
10	18:1	Oleico	78.447	2.248	81.916	73.941	68.518	2.060	72.126	64.787
11	18:1n-7	Cis-vaccenic	1.664	0.437	2.515	0.97	2.738	0.349	3.419	1.807
12	18:2n-6	Linoleic	1.987	0.294	2.478	1.183	1.895	0.520	3.415	1.274
13	18:3n-3	Alfa linolenic	0.644	0.102	0.823	0.478	0.904	0.177	1.261	0.684
14	20:0	Arachidic	0.081	0.026	0.128	0.035	0.076	0.016	0.105	0.05
15	20:1n-11	Gondoic	0.112	0.013	0.137	0.078	0.110	0.017	0.133	0.077
		∑ Saturated [a]	15.014				24.21			
		∑ Monounsaturated [b]	80.340				71.421			
		∑ Polyunsaturated [c]	2.62				2.799			
		Lipid content (%)	48.089	7.817	62.38	33.616	54.192	4.412	62.545	45.248

[a] 12 :0+14 :0+15 :0+16 :0+17 :0+18 :0+20 :0

[b] 16 :1+17 :1+18 :1+18 :1n-7+20 :1n-11

[c] 18 :2n-6+18 :3n-3

In general term, the biplot PCA (Figure 1) revealed a clear-cut distinction between *bataua* and *oligocarpa* varieties, based on fatty acid composition and lipid content. The *bataua* variety contained higher concentrations of oleic, stearic, linoleic, palmitoleic, margaric and 15:0 acids. The *oligocarpa* variety was characterized by a higher concentration of palmitic, cis-vaccenic, alpha-linolenic, myristic, lauric and undetermined 13:0-14:0 acids, and a higher lipid content.

Table 2. Minor Fatty Acids detected in the oil derived from the mesocarp of *Oenocarpus bataua*.

			Western Amazonian *bataua* variety *n*=16 ind.				Guayana region *oligocarpa* variety *n*=22 ind.			
#	Shorthand designation	RT	Mean	±SD	Max	Min	Mean	±SD	Max	Min
1		0,21	0,026	0,025	0,075	0,000				
2	8:0	0,21 /Caprylic	0,058	0,102	0,379	0,000				
3		0,22	0,032	0,030	0,096	0,000				
4	9:0	0,23	0,063	0,103	0,383	0,000				
5	10:0	0,24 /Capric	0,020	0,016	0,058	0,000				
6		0,47	0,059	0,079	0,317	0,005				
7		0,53	0,055	0,040	0,136	0,000				
8		0,57	0,086	0,108	0,429	0,000				
9		1,16					0,031	0,008	0,046	0,014
10		1,45	0,162	0,171	0,664	0,000				
11		1,48	0,123	0,119	0,464	0,000				
12		1,49	0,060	0,076	0,234	0,000				
13		1,49	0,337	0,313	1,207	0,000				
14		2,81	0,025	0,039	0,108	0,000				
15		2,83	0,056	0,051	0,160	0,000				
16	24:0	2,86	0,086	0,082	0,241	0,000				
17		2,89	0,260	0,265	0,927	0,000				

Table 3. Eigenvalues larger than 1.0 and their respective eigenvector loadings.

FAs	PC Axis 1	PC Axis 2	PC axis 3
12:00	-0.266	0.028	0.031
13-14	-0.308	0.055	0.267
14:00	-0.172	-0.266	-0.152
15:00	0.254	0.151	-0.323
16:00	-0.345	0.018	0.112
16:1w7	-0.315	0.142	-0.082
17:00	0.320	0.069	-0.051
17:01	0.062	0.459	-0.363
18:00	0.329	-0.157	0.022
18:1w9	0.334	0.000	-0.135
18:1w7	-0.315	0.106	-0.230
18:2w6	0.053	0.009	0.566
18:3w3	-0.239	-0.114	-0.284
20:00	0.064	-0.518	0.041
20:1w11	0.048	-0.495	-0.111
Lipid content	-0.172	-0.323	-0.399
Eigenvalue	7.717	2.163	1.614
% Variance	48.23	13.52	10.09
% Stored	48.23	61.75	71.84

Discussion

The fatty acid composition of the mesocarp oil of *Oenocarpus bataua* is highly variable along the geographical range of the species. To difference of the former work of Opute (1978) who stated that mesocarp oil fatty acids fingerprints were not enough informative for taxonomic considerations, our study reveals that quantification of fatty acids derived from the mesocarp of *O. bataua* fruits do allow to distinguish intraspecific taxa, as far our sampling can tell.

Variation in mesocarp oil fatty acids composition between *bataua* and *oligocarpa* varieties is in perfect agreement with the botanical evidence (Balick, 1986; Henderson, 1995). Morphological studies have reported a major discontinuity in the distibution of *Oenocarpus bataua* populations, with *bataua* variety occurring in the Amazon valley and *oligocarpa* variety mostly restricted to the Guayanas region. The congruence between morphological and chemotaxonomical data gives us insights about the usefulness of fatty acids as taxonomic markers.

The nature of this biochemical variation might be (*i*) intrinsic to each variety or (*ii*) influenced by the environment, as each variety comes from distinct geological regions of northern South America, the Amazon valley and the Guayana shield. The impact of the environment and specifically of the soils, on the biochemical composition of fruits from wild populations has not been studied. However, the environmental determinism on biochemical constituents has been questioned in some cases (Flamini et al. 2006).

The role of a purely genetic basis explaining this divergence is an alternative hypothesis. Molecular data (AFLP and chloroplastic sequences) have shown a strong genetic differentiation between *bataua* and *oligocarpa* populations (Montufar et al., in prep.). Moreover, these varieties have previously been considered as distinct species based on morphology (Grisenbach & Wendland, 1864 in Balick 1986; Wessels Boer, 1965).

Chemotaxonomic variation between bataua and oligocarpa populations

The knowledge of the natural variation of the fatty acid composition of fruits is important for future programs of domestication of this palm species. The fruits of *Oenocarpus bataua* var. *oligocarpa* were richer in lipid content (mean 54.19%) than those of *bataua* variety (48.08%). However, Lubrano & Robin (1997) and Escriche et al. (1999) reported mean values of 16% and 10.7% for the lipid content from French Guiana (*oligocarpa* variety) and Western Amazonia (*bataua* variety), respectively. These values are much lower than the minimal value obtained in this study from an individual of *bataua* variety (33.6%). Variation in lipid content between studies might be related to the methodologies applied to determine the fat content.

The qualitative composition of these two varieties was in agreement with the fatty acid composition reported in the literature (Balick & Gershoff, 1981). A surprising results of the present analysis was the detection of cis-vaccenic acid in both populations. Although this acid had been previously found at low concentrations in other plant groups (Avato et al., 2003; Mongrand et al., 2005), this is the first time it is reported as a significant component in the oil of *O. bataua*. In particular, cis-vaccenic acid was the third most predominant fatty acid in *oligocarpa* samples.

The saturated fatty acid fraction (mostly palmitic and stearic) was higher for *oligocarpa* (24%) than for *bataua* variety (15%). In particular, the concentration of palmitic acid was twice as greater for *oligocarpa* samples. The saturated fraction for *oligocarpa* variety pointed out in this study was below the value reported by Alemán et al. (2002; 34.47%) from the Upper Orinoco (Venezuelan Guayana), whereas it was similar to the mean values reported by Lubrano & Robin (1997; 23.5%) from an *oligocarpa* population of French Guiana. On the other hand, the low concentration of saturated acids for Amazonian populations (var. *bataua*) had already been noticed by Balick & Gershoff (1981; 16.8%).

The monounsaturated oleic acid was observed to be present in large amounts in *Oenocarpus bataua* mesocarp. The highest oleic fatty acid concentration was observed in *bataua* variety (78 %), in which the total monounsaturated fraction reached 80 %. The *bataua* variety reached values for oleic acid close to 82% for an individual from western Amazonia. The concentration of oleic acid and the total monounsaturated fraction were significantly lower for *oligocarpa* variety (oleic acid 68.51%, monounsaturated 71.33%).

The total polyunsaturated fraction (linoleic, alpha-linolenic) of *Oenocarpus bataua* fruits was fairly similar in *bataua* and *oligocarpa* varieties. However, significant differences among varieties were detected for alpha-linolenic acid. The concentration of linoleic acid was fairly similar in both varieties.

Conclusions

The fatty acid composition of the mesocarp of *Oenocarpus bataua* fruits offers a wide range of biochemical prospects for future plant breeding and selection focused on its oleaginous properties. Future works need to explore the biochemical variation for other wild populations of this species. For example, the fruits of *Oenocarpus bataua* from Chocó forest (Pacific coast of Ecuador and Colombia) are considered by the local people as of lower quality than those of the Amazonia. *O. bataua* populations from the Andes slopes (above 1000 m of altitude) are also interesting populations to be explored by chemotaxonomic markers.

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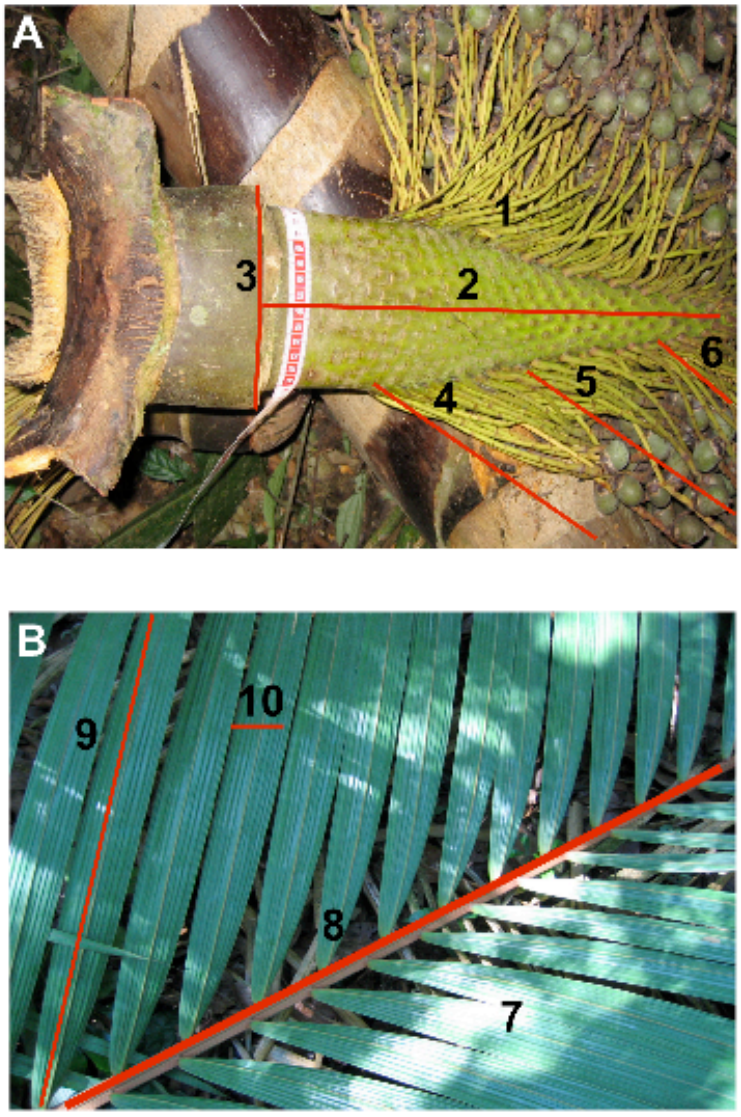
III-*b* Morphological variation among varieties of *Oenocarpus bataua* Mart.

Introduction

Oenocarpus bataua is an arborescent and pinnate-leaved palm widespread in the Neotropical rain forest. It reaches up to 25 m tall and 20-45 cm in diameter. The massive inflorescences can measure more than 1.5-2 m long. Leaves are *ca.* 10 m long and composed of numerous pinnae. Herbarium collections of this large organism are scarce in comparison with its wide geographical range and local abundance. The available herbarium collections of *O. bataua* are composed of fragments of reproductive and vegetative structures and the collection coverage is restricted to few well-inventoried areas. In addition, flowers and fruits are not always present in herbarium collections.

Balick (1986) provided a systematic treatment of the related genera *Oenocarpus* and *Jessenia*. This work —based on herbarium collections and extensive fieldwork— recognized the species *Jessenia bataua* with two subspecies, one from the Amazon valley (subsp. *bataua*) and one from the Guayanas region (subsp. *oligocarpa*). Henderson (1995) reduced *Jessenia* in synonymy of *Oenocarpus* and recognized the two infraspecific taxa as varieties of *Oenocarpus bataua* (var. *bataua* and var. *oligocarpa*). We will use this latter nomenclature throughout this article. The morphological distinctiveness of the varieties was based on six morphological traits: (*i*) color of indumentum on the abaxial surface of pinnae, (*ii*) shape of basal and apical parts of rachilla, (*iii*) position of the triads on the rachilla and consequently the appearance of the infructescences (condensed or spread out); (i*v*) number of rachillae, (*v*) size of the staminate flower, and (*vi*) number of stamens. However, some of the former traits (*v, vi*) exhibit overlapping ranges between varieties (Henderson 1995). Additionally, Henderson (1995) included the appearance of the pinnate leaf (erect or arching) as a morphological difference between var. *bataua* and var. *oligocarpa*.

The morphometry can be a good tool to explore the infraspecific variation within *Oenocarpus bataua*. Morphometry is the quantitative analysis of biological form and its variation (Rohlf 1990, Henderson 2006). To the difference of traditional herbarium systematics, morphometric data comes from distances between landmarks and these are



1 Number of rachillae
2 Floral rachis length
3 Floral rachis circumferance
4 Length of basal rachilla
5 Length of middle rachilla
6 Length of apical rachilla
7 Number of pinnae
8 Foliar rachis length
9 Length of middle pinna
10 Width of middle pinna

Figure 1. Inflorescence (a) and leaf (b) of Oenocarpus bataua showing where measurements were made.

mostly measured directly on the specimen. Moreover, morphometry introduces multivariate or univariate statistical techniques to analyses morphologic data.

Morphometric studies focused on the natural variation of Neotropical palms developed considerably during the last years. Morphometric techniques were successfully applied to explore the natural variation of *Hyospathe* (Henderson 2004), *Synechanthus* (Henderson & Ferreira 2002), *Reinhardtia* (Henderson 2002) and *Calyptrogyne* genera (Henderson 2005). In contrast, morphometry was less informative to delimit infraspecific taxa within a species complex like *Geonoma stricta* (Henderson & Martins 2002) and *Geonoma cuneata* (Borchsenius 1999).

This study proposes a new approach to explore the infraspecific variation of morphological traits in *Oenocarpus bataua*. Due to the limitations of herbarium collections for this palm species, the present study is based on the documentation of news traits taken *in situ* from natural populations.

Material and methods

Thirteen quantitative characters (Table 1) were measured or counted directly of natural populations of *Oenocarpus bataua* from Peruvian Amazonia (var. *bataua*) and French Guiana (var. *oligocarpa*). No specimens from intermediate areas were examined. Flowers and fruits traits were not included in this analysis due to absence of these phenological states during the fieldwork, and all characters were recorded from reproductive adults. Five qualitative characters were recorded in the field but not included in the statistical analyses (Table 2). Fieldwork was carried out during the months of July and August 2005 for *bataua* population from Peruvian Amazonia (localities of Jenaro Herrera and Intuto), and November and December 2004 for *oligocarpa* population from French Guiana (localities of Saül and Regina). Differences of the characters between *bataua* and *oligocarpa* were analyzed using an univariate analysis (Wilcoxon test) as implemented by JMP software (1989-94 SAS Institute).

Stem circumference was measured at breast high. The length and width of middle pinna and the number of pinnae were recorded from leaves selected from adult individuals. Prophyll and peduncular bract found on the grown were measured. The values of length of

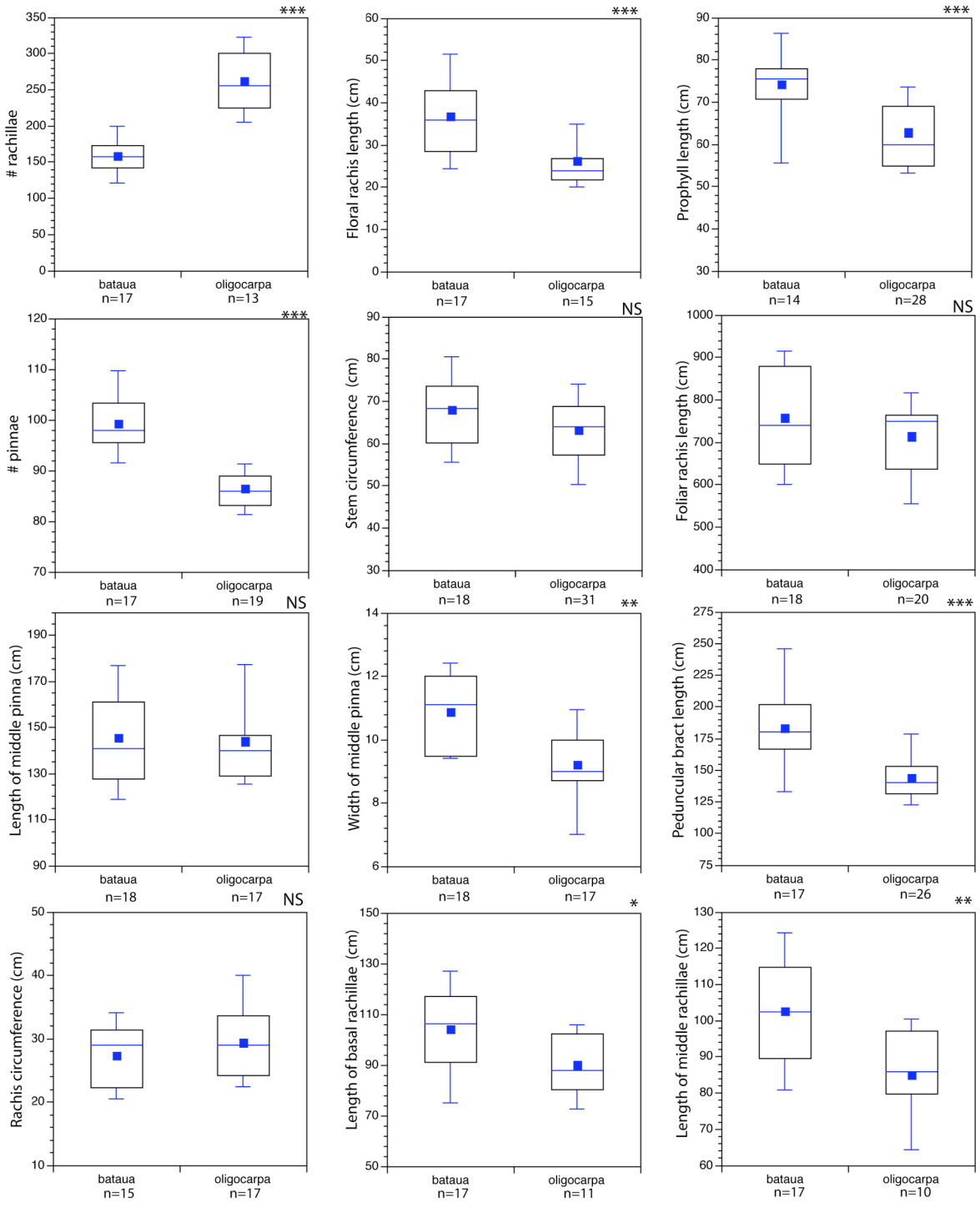


Figure 2. Box-plots summarizing the variation of 12 characters for two varieties of *Oenocarpus bataua* (*bataua, oligocarpa*). Boxes show the 25th and 75th percentiles, median and mean (solid square); whiskers show the min/max values. P values is shown on the upper right corner of each box-plot: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, NS $p > 0.05$. n = number of samples.

basal, middle and apical rachillae correspond to the average values of ten rachillae for each position. The locations of inflorescence (reproductive) and leaf (vegetative) variables measured are shown in Fig. 1.

Results and Discussion

Table 1 and figure 2 shows the mean, median and percentiles of twelve of the thirteen variables studied. Significant statistical differences ($p < 0.05$) were detected for eight of thirteen variables analyzed. The recognition of a morphological divergence between var. *bataua* and var. *oligocarpa* as proposed by Balick (1986) is supported by this study. Moreover, our results reveal a new set of morphological traits –different from those formerly used by Balick (1986) and Henderson (1995)- that might be used to discriminate between *bataua* and *oligocarpa* specimens.

Six of eight morphological characters derived from the inflorescence discriminated clearly the two varieties. *Bataua* was well separated from *oligocarpa* by its number of rachillae, floral rachis length, the length of the prophyll and peduncular bract and the length of basal and middle rachillae. The number of rachillae has previously been used by Balick (1986) to separate the varieties. This work supports the use of the number of rachillae as a strong character to distinguish *bataua* from *oligocarpa*. *Oligocarpa* specimens exhibited a greater number of rachillae (mean 261; maximal 322; minimal 201) than *bataua* (158; 212; 98).

Unlike characters derived from the inflorescence, vegetative traits (stem and leaves) were less informative to differentiate varieties. Only two of five vegetative characters (number of pinnae, width of middle pinna) exhibited significant differences between varieties. Particularly, the number of pinnae (bataua: mean= 96; oligocarpa: mean= 86) shows a clear differentiation between *bataua* and *oligocarpa* varieties.

Vegetative characters seem to be more sensitive to local conditions, particularly edaphic conditions. For example, the stem circumference was observed to be highly variable in function of the soil type in the locality of Jenaro Herrera (Peruvian Amazonia). Individuals growing on poor and sandy soils had thin stems; whereas specimens from rich and clay

soils from the same locality showed wider stems. This also applies to some degree to other vegetative features like the length of the leaf and pinnae.

Table 1. Quantitative characters used in this study.

	Oenocarpus bataua Martius	
	bataua	*oligocarpa*
	mean±standar deviation	mean±standar deviation
	n= samples number	*n*= samples number
TRAIT (unit)		
Number of rachillae (#)	158.41±28.02	261.46±43.04
	n=17	*n*=13
Floral rachis length (cm)	36.81±9.5	26.2±7.83
	n=17	*n*=15
Prophyll length (cm)	74.25±11.30	62.8±8.7
	n=14	*n*=28
Number of pinnae (#)	95.94±15.32	86.36±4.38
	n= 18	*n*=19
Stem circumference (cm)	67.97±8.87	63.17±9.09
	n=18	*n*=31
Foliar rachis length (cm)	757.05±126.1	713.25±105.64
	n=18	*n*=20
Length of middle pinna (cm)	145.55±22.44	143.94±20.91
	n=18	*n*=17
Width of middle pinna (cm)	10.86±1.36	9.21±1.54
	n=18	*n*=17
Peduncular bract length (cm)	183.26±47.19	143.84±21.82
	n=17	*n*=26
Rachis circumference-	27.30±5.55	29.32±6.02
at insertion bract level (cm)	*n*=17	*n*=15
Length of basal rachilla (cm)	104.17±19.59	89.81±13.85
	n=17	*n*=11
Length of middle rachilla (cm)	102.68±17.27	84.90±13.12
	n=17	*n*=10
Length of apical rachilla (cm)	84.49±13.09	78.77±12.89
	n=16	*n*=11

Some qualitative characters might be useful to discriminate *bataua* and *oligocarpa* varieties (Table 2). Particularly, the fibrous petiole base (a greater production of fibers for oligocarpa) and the disposition of the triads on rachillae (triads born on proximal 1/3 of rachillae for *oligocarpa* and 1/2 of the rachillae for *bataua*) exhibited a consistent variation between varieties. Conversely, qualitative characters described in the literature like the color of the indumentum and shape of rachillae (Balick 1986) were not clearly differentiated in the field. Color of the indumentum would be more informative for to anatomical study than in the field; and shape of rachillae showed a strong variation among

and inter-individuals. Henderson (1995) uses the appearance of the leaf (tending to be arching for oligocarpa and erect for bataua) to separate these populations. As the former features it was not easily evaluated in the field.

Table 2. Qualitative characters used to discriminate *bataua* and *oligocarpa* varieties (Balick* 1986, Henderson° 1995), and states observed in Peruvian (*bataua*) and Guianan (*oligocarpa*) populations in this study.

	bataua	*oligocarpa*
Color of indumentum*	whitish	whitish
Rachilla shape*	Not evaluated	Not evaluated
Disposition of the triads on rachillae*	Proximal 1/2	Proximal 1/3
Leaves (arching or erect)°	erect	erect
Fibers on petiole base	moderate	very abundant

The use of quantitative characters, particularly from inflorescence allowed detecting a morphological differentiation between *bataua* and *oligocarpa* populations. The significant differentiation reported here provides support for the recognition of these infraspecific taxa. In addition to the former variables used by Balick (1986), this study report new reproductive and vegetative characters supporting this differentiation.

III-*c* *Oenocarpus bataua* — Rediscovering a source of high oleic vegetable oil from Amazonia

MANUSCRIT ACCEPTÉ SOUS RÉSERVE DE RÉVISIONS MINEURES dans *FOOD CHEMISTRY*

Rommel Montúfar, Andréina Laffargue, Jean-Christophe Pintaud, Serge Hamon, Stéphane Dussert

Abstract

The fatty acid (FA) composition of *Oenocarpus bataua* oil from 38 samples collected over a large geographical range (i.e. French Guiana and Peru) was analyzed. Fifteen fatty acids were obtained from the mesocarp of this palm species. Oleic acid (mean 72.69%) and palmitic acid (18.12%) were the predominant FAs. Other minor FAs were cis-vaccenic acid (2.28%), linoleic acid (1.93%), stearic acid (1.74%), palmitoleic (0.89%) and alpha-linolenic acid (0.79%). Dry mesocarp was found to have a 51.6% lipid content. *O. bataua* oil is highly nutritional due to its high oleic acid content and low level of saturated fatty acids.

Keywords: Amazonia, Arecaceae, fatty acids, *Oenocarpus bataua*

1. Introduction

The search for new sources of vegetable oils has been a topic of keen interest over the last 20 years. It is of particular interest for developing countries where edible oil consumption is mainly based on oils that can be detrimental to human health (saturated oils), such as African palm oil and coconut oil (Srinivasan, Irz, & Shankar, 2006). Palm fruits are one of the main sources of oils and fats, but few palm species have been exploited. The Amazonian region holds more than 150 palm species (Henderson, 1995), some of which (e.g. *Oenocarpus bataua, O. bacaba, Mauritia flexuosa, Attalea maripa, A. speciosa, Bactris gasipaes*) represent a great potential as source of edible oils. The genus *Oenocarpus*, and particularly the species *O. bataua* (formerly *Jessenia bataua, J. polycarpa,* or *J. oligocarpa*), has been described as a promising palm for the Amazonian

region due to the oil content of its fruits (Balick, 1981; Balick, 1988; Johnson, 1996; Lleras & Coradin, 1988).

Oenocarpus bataua (locally known as ungurahui, seje, patawa, coroba) is a canopy or subcanopy palm (up to 35 m in height, Miller, 2002) whose distribution range includes the northern half of South America, including Panama and Trinidad. It is a monoecious species that is fairly abundant in the Amazonian forests and sometimes forming oligarchic populations on waterlogged soils (Kahn & Granville, 1992).

Oenocarpus bataua is one of the most useful plants for indigenous communities in Amazonia. An abundant ethnobotanic literature describes its uses in the Amazon region (Balick, 1986; Balick & Gershoff, 1981; Borgtoft Pedersen & Balslev, 1993; Macía, 2004). The fruits are mainly used as a source of edible oil and a milk-like beverage. Oil is extracted by boiling the fruits and collecting the lipidic supernatent (Balick, 1986). The milk-like beverage, locally called "chicha" or "wine of seje", is an important source of calories and protein in the indigenous diet (Balick & Gershoff, 1981). The fruits of *O. bataua* and their derivatives (pulp, beverages, oil) are sold in regional Amazonian markets. In Cayenne (French Guiana) and Iquitos (Peru), small local industries produce ice-cream with the mesocarp of this palm. *O. bataua* oil is also sold for medical purposes (hair tonic) throughout the Ecuadorean Amazonian region (Borgtoft Pedersen & Balslev, 1993), and even in phytotherapy shops in major cities like Quito and Guayaquil.

Few studies have focused on the fatty acid (FA) composition of *Oenocarpus bataua* oil despite its potential as a source of vegetable oil. In 1981, Balick and Gershoff reported the first fatty acid composition using gas chromatography. Subsequent experiments were performed by Lubrano and Robin (1997), and Alemán *et al*. (2002). These few studies were based on a small number of samples, and consequently, they were not representative of the overall geographical variation of this species. In addition, a very low number of fatty acids (six or seven) were detected in these studies, suggesting that the sensitivity of the methods used was not optimal. Finally, conflicting results were reported on percentages of FAs (e.g. oleic acid ranged from 40 to 70%).

The aim of the present study was thus to gain further insight into the FA composition and lipid content of *Oenocarpus bataua* mesocarp. We surveyed a broad geographical area

that includes several populations and environments in Peru and French Guiana, in order to highlight possible variations in fruit fatty acid composition and lipid content of this palm species. For comparison purposes, we include the FA composition and lipid content of two Amazonian palm species that are phylogenetically related to *O. bataua*, i.e. *O. bacaba* and *Euterpe precatoria*. The FA composition is discussed in terms of human consumption and nutrition.

2. Materials and methods

Twenty-two and 16 wild *Oenocarpus bataua* individuals were selected from different localities in French Guiana and Peru (Loreto), respectively. The geographic distance among individuals varied between 1-20 km in French Guiana and 1-10 km in Peru. We harvested 10 ripe fruits (dark purple and soft to the touch) directly from the infructescence of each individual selected. The fruits were dried with abundant silica gel and transported in plastic bags. The field work was carried out in February-March 2004 in Peru, and November-December 2004 in French Guiana.

For each individual, the dry mesocarp was isolated mechanically from 5-8 fruits. The mesocarp was reduced to a fine powder using an analytical grinder (IKA A10, IKA® Germany). This material was divided into three samples (replicates) of 0.3 g for fatty acid analyses, and 0.2 g for water content analyses. Total lipids were extracted from the 0.3 g samples using a modified Folch method (Folch, Less, & Stanley, 1957) with methylene chloride replacing chloroform. Lipid content was determined gravimetrically after complete solvent evaporation under a nitrogen stream at 40°C. The mesocarp lipid content was measured in triplicate for each individual of *Oenocarpus bataua* (n=38) using a totally random experimental design. One sample of *O. bacaba* and *Euterpe oleracea* was included in this analysis. The lipid content was expressed on a dry weight basis after measurement of the powder water content. The water content of samples was estimated by desiccation of powders in an oven at 105°C overnight.

Fatty acid methyl esters (FAMEs) were prepared according to the NORME NF EN ISO-5509 (AFNOR, 2000). Roughly, lipid extracts were first saponified with 4 ml of a 0.5 M methanolic solution of sodium hydroxyde at 90°C for 10 min and then methylated with 5 ml of 14% BF_3 methanolic solution at 90°C for 3 min. GC analyses were performed using

an HP 5890 system with flame ionisation detection (FID). A Famewax capillary column (RESTEK, France), 30 m x 0.25 mm x 0.25 µ was used. The analyses were carried out in program temperature mode from 185°C to 225°C at 4°C/min and then in the isothermal mode for 10 min at 225°C**.** Helium was used as carrier gas at 40 $cm.s^{-1}$. Both injector and detector were at 230°C. FAMEs were identified by comparing their retention times with those of the fatty acid methyl ester standards (Sigma) and were quantified as percentages of fatty acids over total fatty acids (w/w). The fatty acid composition was analyzed in triplicate for each individual of *Oenocarpus bataua* (n=38), *O. bacaba* (n=1) and *Euterpe oleracea* (n=1).

3. Results and discussion

Fifteen FAs (Table 1) from mesocarp of *Oenocarpus bataua* were identified in this study, while only six or seven FAs were reported in previous works (Alemán et al., 2002; Balick & Gershoff, 1981; Lubrano & Robin, 1997). Therefore the present data currently represents the most detailed description of the FA composition reported for *O. bataua.* Oleic acid (72.69%) followed by palmitic acid (18.12%) were the major components and both account for approximately 90% of total FAs. Other minor FAs were cis-vaccenic acid (2.28%), linoleic acid (1.93%), stearic acid (1.74%), palmitoleic acid (0.89%), alpha-linolenic acid (0.79%), and very low amounts of 15:0 acid (0.27%), gondoic acid (0.11%), undetermined 13:0-14:0 acid (0.1%), myristic acid (0.09%, Table 1). Cis-vaccenic acid, which has not been recorded in previous studies of the species, was the third most predominant FA detected in our analyses. *Cis*-vaccenic acid has already been found at low concentrations in other plant families (Avato, Pesante, Fanizzi, & Santos, 2003; Mongrand, Badoc, Patouille, Lacomblez, Chavent, & Bessoule, 2005; Reiter, Lechner, & Lorbeer, 1998).

Values obtained in previous studies concerning the predominant FAs of *Oenocarpus bataua* are conflicting (Alemán et al., 2002; Balick & Gershoff, 1981; Lubrano & Robin, 1997). For example, the percentage of oleic acid reached 77.7% of the total FAs reported by Balick and Gershoff (1981), whereas Alemán et al. (2002) detected only 46%. The FA composition of *O. bataua* oil reported by Alemán et al. (2002) from the Upper Orinoco

Table 1. Fatty acids composition of oil derived from the mesocarp of *Oenocarpus bataua* and other palm species. Values are expressed as percentages of total fatty acids.

		O. bataua n=38		*O. bataua* n=12	O. bataua n=1	*O. bataua* n=1	O. bacaba n=1	*Euterpe oleracea* n=1	**Elaeis guineensis* (mesocarp)	**Cocos nucifera*	**Mauritia flexuosa*	**Olea europaea*
		this study[a]		Balick & Gershoff 1981	*Aleman* et al. *2002*	Lubrano & Robin 1997	*this study*	this study				
		Peru /French Guiana		Amazon	Venezuela	French Guiana						
Fatty acids	Fatty acid names	% Mean±SD	% Min/Max	% Mean±SD	%	%						Min/Max
12:0	Lauric	0.01±0.01	0 / 0.03				0.18	0.54	0.1	50		
13:0-14:0	Undetermined 2	0.10±0.06	0 /0.19				0.26	0.24				
14:0	Myristic	0.09±0.05	0.03 / 0.29		0.16		0.59	0.65	0.9-1.1	16		
15:0		0.27±0.08	0.14 / 0.50				0.63	0.07				
16:0	Palmitic	18.12±5.58	9.68 / 25.96	13.2±2.1	28.56	21	32.27	28.48	43.1-45.3	6.5	17.3-23.7	7.4-14.3
16:1ω7	Palmitoleic	0.89±0.37	0.31 / 1.61	0.6±0.2		1	1.32	1.69	0.1-0.3		0.3-0.7	0.9-3.0
17:0	Margaric	0.061±0.01	0.09 / 0.03									
17:1		0.07±0.01	0.11 / 0.05									
18:0	Stearic	1.74±0.79	0.87 / 3.50	3.6±1.1	5.75	1.5	2.75	2.16	4.0-4.8	1	1.4-2.0	3.5-4.8
18:1ω9	Oleic	72.69±5.39	64.78 / 81.91	77.7±3.1	46.06	70	40.82	47.32	38.4-40.8	18.2	70.7-76.5	63.3-81.5
18:1ω7	*cis*-vaccenic	2.28±0.66	0.97 / 3.41				2.01	2.44				
18:2ω6	Linoleic	1.93±0.43	1.18 / 3.41	2.7±1.0	18.04	4	9.78	9.95	9.4-11.1	1	1.9-2.1	5.1-15.5
18:3ω3	Alpha-linolenic	0.79±0.19	0.47 / 1.26	0.6±0.4	0.68	tr	1.93	4.39	0.1-0.4		1	
20:0	Arachidic	0.07±0.02	0.12 / 0.03			2	0.48	0.08	0.1-0.4			1.2-2.6
20:1ω11	Gondoic	0.11±0.01	0.07 / 0.13				0.13					
Others		0.65±1.3	0.00 / 5.9	1.6							0.6-0.8	

n = number of independent samples; tr= trace amounts; *= from Ucciani 1995; blank cells = FAs not detected/not reported

a values are means ± SD for triplicate analys

region (Venezuela) was closer to *O. bacaba* and *Mauritia flexuosa* than to *O. bataua*. The misidentification of the material analyzed (e.g. particularly between *O. bacaba* and *O. bataua*) or the inclusion of interspecific hybrids (*O. bataua* × *O. bacaba*) could explain these outlier values. The percentage of FAs reported by Balick and Gershoff (1981) and Lubrano and Robin (1997) fell within the biochemical variation observed in our data. For instance, the 13% and 21% of palmitic acid reported by Balick and Gershoff (1981) and Lubrano and Robin (1997), respectively, are within the 10-26% range described in this study.

Edible oil derived from *Oenocarpus bataua* fruits has a great potential as a new source of monounsaturated oils. The main monounsaturated acid in *O. bataua* oil was oleic, with around 73% of total fatty acids. The high oleic content of *O. bataua* oil is comparable to olive (~75%) and "high oleic" sunflower oil (>80%; Rönicke, Hahn, & Friedt, 2005). These former vegetable oils have a substantial nutritional value, since high intake of monounsaturated acids is considered to be a dietary factor involved in lowering cholesterol, and the incidence of coronary heart diseases and hypertension (Delplanque, 2000; Weisburger, 2002).

The oil of *Oenocarpus bataua* fruits, with ~20% of stearic/palmitic acids and 71% oleic acid, is close to the composition recommended for an ideal vegetable oil (25% and 60%, respectively, see Martin, 2001 and World Health Organization, 2003). However, *O. bataua* oil is poor in linoleic (2%) and alpha-linolenic acids (0.8%) compared with the recommended values of 12% and 2%, respectively. This deficit in polyunsaturated FAs might be improved with agronomic breeding and selection. Furthermore, the deficit of some FAs could be enhanced by using other sources of vegetable oil like *Oenocarpus bacaba* and *Euterpe oleracea*, which are particularly rich in linoleic and α-linolenic acids (Table 1). *O. bataua* and *O. bacaba* grow together in the tropical forest of the Guayanas region. A mixed oil derived from these two palm species would produce a vegetable oil that is rich in oleic acid and linoleic acid. In eastern Amazonia, *O. bataua* oil could be processed together with the fruits of *Euterpe oleracea* to offset this deficit in linoleic acid and alpha-linolenic acid.

The mesocarp of *Oenocarpus bataua* is rich in lipids, i.e. 51.6 % of its dry weight (Table 2). Compared with the other two species included in this study, *O. bataua* had a higher

lipid content than *O. bacaba* and *Euterpe oleracea*, with only 3.1% and 7.2%, respectively. Studies quantifying the lipid content of *O. bataua* fruits are scarce (Balick & Gershoff, 1981; Lubrano & Robin, 1997), and the results of these latter studies cannot be directly compared because the methods differed.

Table 2. Lipid content of mesocarp of three Amazonian palms.

	Lipid content Mean±SD
Oenocarpus bataua (n=40)	51.61±6.62
Oenocarpus bacaba (n=1)	3.16
Euterpe oleracea (n=1)	7.24

Oenocarpus bataua is an under-tapped source of high-quality oleic oil from the Amazonian forest. Its beneficial FA composition and high protein content (Balick, 1988) could help to improve the nutritional conditions for the native people in this region. In addition, oil extracted from *O. bataua* seeds (kernel oil) is rich in lauric oils (Salazar, Belén, Jiménez, & Pino, 2004) and could be utilized simultaneously. The long-term conservation of this resource is the main concern for its sustainable use. During the fruiting season, hundreds of adult individuals of this palm are cut-down in order to gather their infructescences. The impact of this destructive extractivism has not been precisely evaluated, but natural populations of this species could be seriously depleted in the vicinity of Amazonian villages. Such alteration of natural populations could result in loss of valuable biological diversity, which is crucial for developing a domestication program for *O. bataua*.

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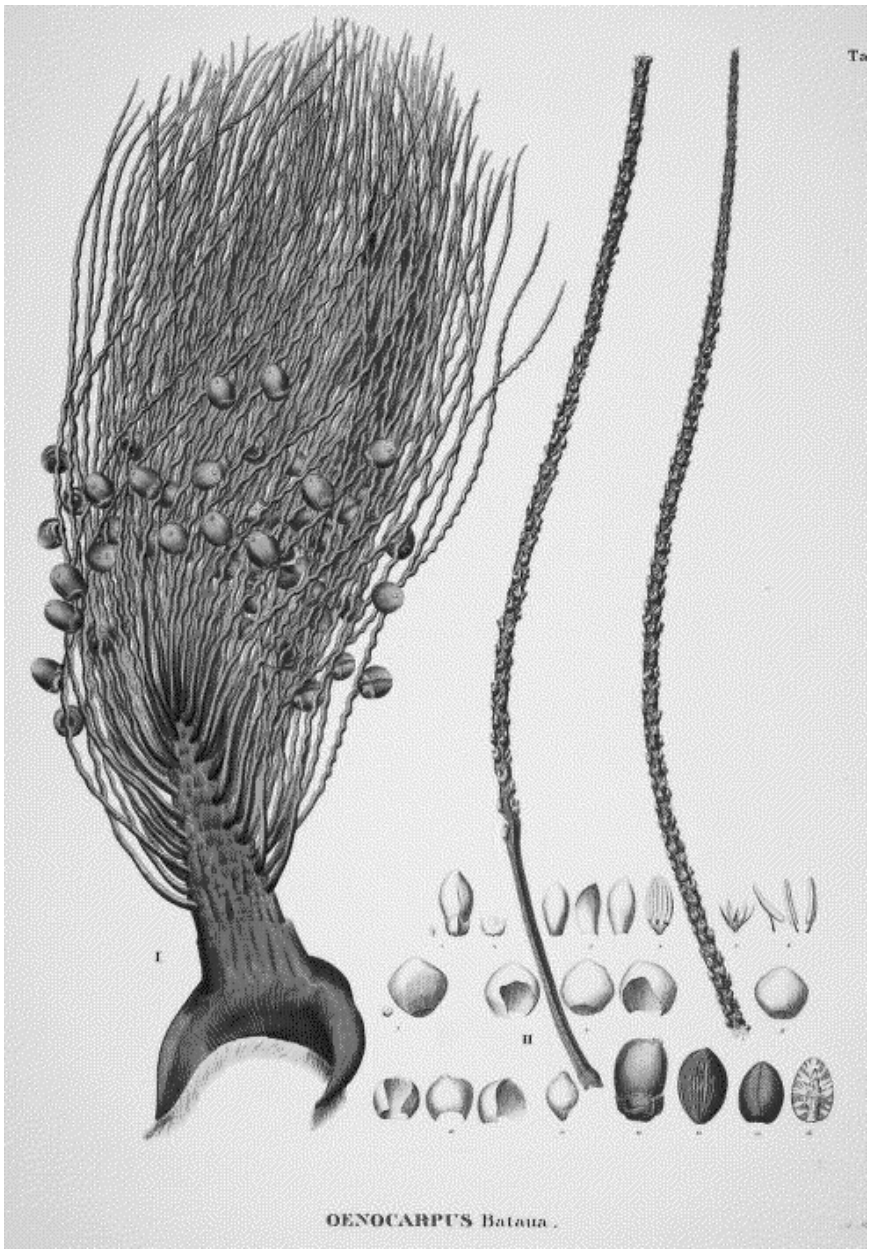
IV

STRUCTURE POPULATIONNELLE ET DIFFERENTIATION INTER-POPULATIONNELLE



IV-*a* Genetic structure of three ecologically differentiated populations of *Oenocarpus bataua* Mart. (Arecaceae), in Western Amazonia

Introduction

Western Amazonia is one of the most biologically diverse environments of the world. Particularly, the Napo region, which is shared by Colombia, Ecuador and Peru, harbors one of the highest plant species richness of the region (Valencia *et al*. 1994; Vásquez-Martínez 1997; Duivenvoorden & Lips 1998). This region is seriously threatened by human pressure (Myers 1988); approximately, 1% of its area (100.000-200.000 ha) is deforested every year (Sierra 2000). In addition, the quality of the remnants forest decline due to the fragmentation and human interference.

The erosion of the genetic diversity is a major concern for the biological conservation of the Napo rainforests. The genetic pool of thousand of plant species could be severely reduced at medium to long-term if the annual rate of deforestation remains constant or increases. From an agronomic point of view, the devastation of the Napo rainforest would result in the loss of the genetic variability of many plant species with potential as new crops. Palm trees, and particularly, *Oenocarpus bataua* is an example of such concerns, as its genetic diversity is threatened by deforestation and destructive extractivism in this region.

O. bataua is a monoeceous, out-crossed, non-domesticated palm species largely distributed in the tropical regions of northern South America, Panama and Trinidad. Particularly, it is highly abundant in Western Amazonia (Montufar & Pintaud 2006, Vormisto *et al.* 2004). This is a wide-ranging palm species displaying different ecological preferences over its geographical range. In the westernmost area of the Napo region, *O. bataua* grows on well-drained soils (*i.e*., *terra firme*, Borgtoft Pedersen & Balslev 1993), whereas in the easternmost extreme it grows on poorly drained soils (*i.e.,* waterlogged areas, swamps, Kahn & Granville 1992). In a geographically intermediate area of the Napo region, this palm grows indistinctively on well-drained or poorly drained soils (Montufar & Pintaud 2006). Ecologically, its large inflorescences and fruits rich in calories favor the establishment of multiples interactions with pollinators and dispersers (Kahn & Granville 1992, Peres 1994, Stevenson *et al*. 2000). Moreover, it is an important

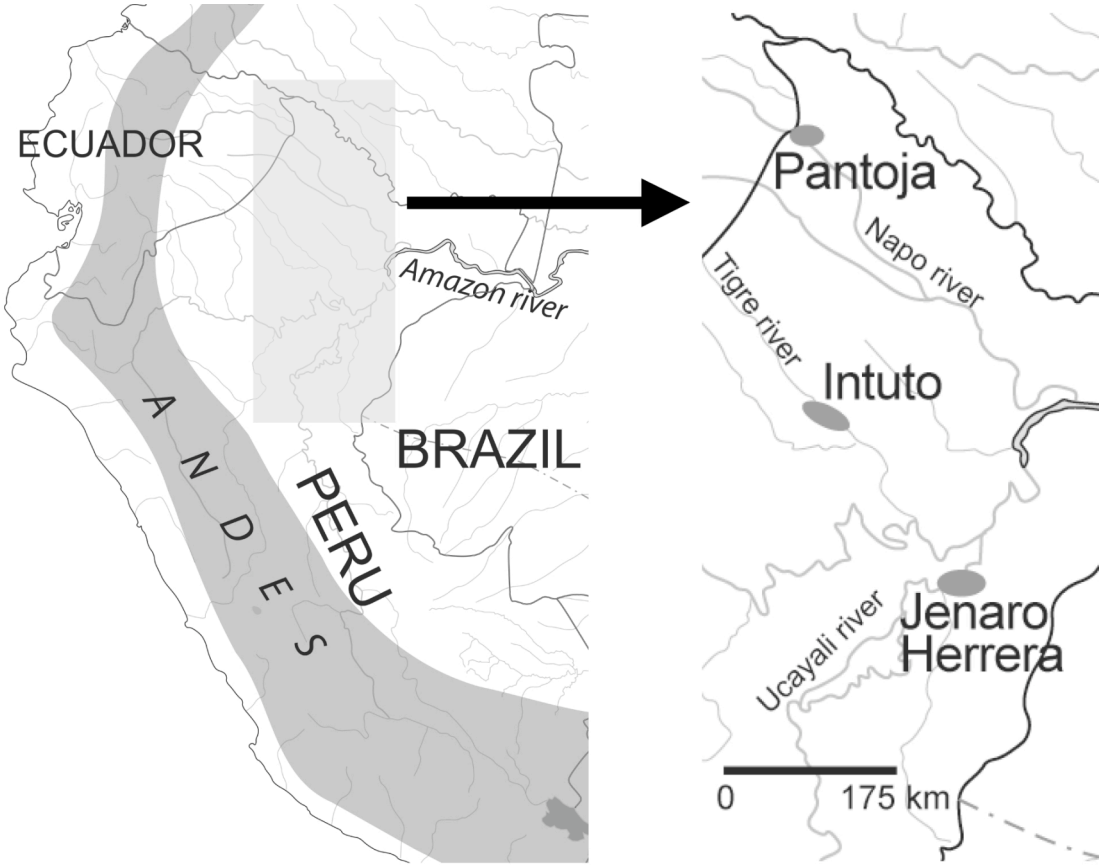


Figure 1. Study area and sampling locations

producer of organic matter in the Amazonian forest (Kahn & Granville 1992). The fruits of *O. bataua* are consumed by indigenous communities and householder of the Amazon region as source of vegetal oil and proteins (Balick 1981). In spite of its ecological and economic importance, no study exploring the genetic diversity of *Oenocarpus bataua* at the intraspecific level has been conducted to date.

As a first approach to understand the genetic diversity of *Oenocarpus bataua*, we analyzed the molecular variability within and among three wild populations from Western Amazonia representing the ecological diversity of the species in the region, using eight microsatellite loci.

Materials and methods

Samples

A population was defined as a group of individuals growing together within a limited area (5-15 km^2), and potentially interfertile, Each population was located 200-400 km apart in the Peruvian Amazon (Loreto region, Figure 1). No natural or human barriers separate these populations. The first population was located in Pantoja, close to the border with Ecuador, on Upper Napo river. In this locality, *Oenocarpus bataua* individuals grow abundantly on well drained soils (slopes/hills habitat). Thirty individuals were collected in Pantoja. The second population was located in Jenaro Herrera, lower Ucayali river basin. Thirty individuals from this locality were collected on swampy areas (swamp habitat). The third population comes from an area in between the former two, close to the locality of Intuto, on Rio Tigre. In this locality 16 and 14 individuals of *O. bataua* growing on slopes/hills and swamps, respectively, were sampled. The individuals collected from each population were separated by at least 100 m from each other. Statistical significance of the ecological specialization of the three populations with respect to the topography and drainage conditions has been reported previously (Montufar & Pintaud 2006).

DNA extraction and Molecular markers

For genetic analyses, fresh material from the young or mature leaves were collected and dried with abundant silica gel. Genomic DNA was extracted from the dried tissues with DNeasy plant mini kit (Qiagen), following manufacturer's instructions. Population genetic structure was assessed using 8 microsatellite loci for *Oenocarpus bataua*, previously

described by Montufar *et al*., (in press). PCR reactions were performed in a 15 µl total volume, with 125 µM dNTP mix, 1 U of *Taq* DNA polymerase, 1X PCR reaction buffer (Colorless Go-*Taq*, Promega), 1 mM $MgCl_2$, 0.04 µM forward 5'M13 primer, 0.1 µM reverse primer, and 0.1 µM M13 fluorescent labelled (IRD-700) primer. The thermal cycling profile was 2 min of initial denaturation at 96°C followed by 35 cycles of 95°C for 30 s, 55°C annealing temperature for 30 s, and 72°C elongation step for 45 s. A final 10 min extension step at 72°C was added. PCR products were diluted 1/25 with pure water, and latterly 2 µl of the former dilution was diluted into 10 µl of foramide high definition plus 2 µl of molecular marker. Amplified products were detected with an ABI Prism® 3130xL Genetic Analyzer (Applied Biosystems). Allelic patterns were coded using GENEMAPPER 3.7 (Applied Biosystems).

Statistical analyses

The observed heterozygosities (H_O), the gene diversity (H_E), the number of alleles (N_A) and the linkage equilibrium among loci were calculated with GENEPOP (Raymond & Rousset 1995). The excess or deficit of heterozygotes (F_{IS}, Weir & Cokerham 1984) was computed for each microsatellite locus within each population (n=3) and on whole data set with 999 bootstraps using the software GENETIX (Belkhir *et al*. 2001).

Differentiation among populations was estimated with a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al*., 1992) carried out with the software Arlequin, version 1.1 (Schneider *et al*. 1997). Significance levels for the F_{ST} values were assessed with 1023 permutations. The AMOVA was carried out on two different rearrangements of the dataset: (a) by populations (Pantoja, Intuto, Jenaro Herrera, n=90), (b) by habitat (slopes-hills and swamps, n=90). Due to the presence of the species in both habitats in Intuto, an AMOVA was carried out by habitat for this population only (slopes-hills habitat n=16; swamp habitat n=14; Table 2).

Population structure was evaluated with the software STRUCTURE version 2.1 (http://pritch.bsd.uchicago.edu; Pritchard *et al*. 2000). The optimal number of populations (*K*) was searched from K=1 to K=10 with ten replicate runs each. We computed a model with 200.000 Markov chain Monte Carlo (MCMC) iterations and a burning length of 200.000. Parameter settings were: an admixture model, alleles frequencies correlated

among populations, non-informative priors. The detection of the optimal number of clusters (K) values was calculated with ΔK as proposed by Evanno *et al*. (2005). A UPGMA analysis was carried out with whole data set using shared alleles index as implemented by Powermarker 3.25 (Liu & Muse 2005).

Results

Genetic diversity

All microsatellites analyzed were highly polymorphic for each population studied (Table 1). The polymorphism of each locus for the whole dataset (n=90) ranged from seven to 24 alleles, with a mean of 14 alleles. The populations of Jenaro Herrera and Intuto displayed a higher allele number (mean values of eleven and ten, respectively) than Pantoja (mean value of seven). At the population level, no significant departure from the Hardy-Weinberg equilibrium was detected as evidenced by F_{IS} values, to exception of Ob20 and Ob03 for Jenaro Herrera (F_{IS} = 0.161, F_{IS} = 0.178, respectively), and Ob17 for Intuto (F_{IS} = 0.128, Table 1). For the entire data set, four loci (Ob20, Ob14, Ob03, Ob19) showed significant departure from the Hardy-Weinberg equilibrium. The test of linkage equilibrium found a single pairwise comparison with a significant deviation from zero (Ob06-Ob14 for Intuto, P =0.01).

Variance partitioning

Pairwise F_{ST} values revealed a low but significant differentiation among populations (Pantoja *vs.* Jenaro Herrera 0.129 P<0.001; Pantoja *vs.* Intuto 0.096 P<0.0001; Intuto *vs*. Jenaro Herrera 0.089 P< 0.001). F_{ST} values even lower but still significant were found between habitats (swamp *vs.* slopes 0.061 P<0.001). Within Intuto, no association between habitat and the genetic structure was detected ($F_{ST=}$ 0.001).

The AMOVA analyses (Table 2) reveals that 89.43% of the genetic variation was observed within populations, and only 10.57% among populations (n=3, Pantoja, Intuto, Jenaro Herrera). Classifying the individuals by their habitat (n=2, swamp and slopes/hills), 93.81% of the genetic variation was reported within habitats, and only 6.19% among habitats. When considering only Intuto population and analyzing the samples by habitat, almost the totality of the variance (99.8%) was reported within population.

Table 1. Description of microsatellite loci (Montufar *et al.* in press) used in this analyses. H_E expected heterozygosity; H_O observed heterozygosity ; F_{IS} fixation Index (Weir & Cockerham 1984) ; N_A Allele number ; ** / *** very significant / significant departure from the Hardy-Weinberg equilibrium after 1000 permutations.

LOCUS		Pantoja n=30	Intuto n=30	Jenaro Herrera n=30	Pooled dataset n=90
Ob01	H_E	0,833	0,718	0,912	0.889
	H_O	0,828	0,867	0,933	0.876
	F_{IS}	0,041	-0,174	0,009	0.020
	N_A	11	12	19	22
Ob20	H_E	0,443	0,709	0,5	0.734
	H_O	0,433	0,69	0,467	0.528
	F_{IS}	0,055	0,063	0,161**	0.286***
	N_A	4	7	7	9
Ob06	H_E	0,745	0,847	0,826	0.861
	H_O	0,8	0,926	0,867	0.850
	F_{IS}	-0,04	-0,055	0,004	0.019
	N_A	8	10	14	15
Ob15	H_E	0,779	0,717	0,804	0.816
	H_O	0,833	0,759	0,759	0.772
	F_{IS}	-0,035	-0,023	0,116	0.06
	N_A	6	10	12	14
Ob14	H_E	0,401	0,537	0,613	0.600
	H_O	0,379	0,586	0,536	0.511
	F_{IS}	0,089	-0,055	0,118	0.154**
	N_A	3	6	3	7
Ob03	H_E	0,707	0,76	0,835	0.812
	H_O	0,7	0,7	0,724	0.707
	F_{IS}	0,044	0,113	0,178***	0.135**
	N_A	7	9	10	12
Ob19	H_E	0,718	0,818	0,87	0.888
	H_O	0,767	0,857	0,897	0.839
	F_{IS}	-0,033	-0,012	-0,016	0.061*
	N_A	7	17	16	24
Ob17	H_E	0,656	0,798	0,831	0.828
	H_O	0,655	0,724	1	0.793
	F_{IS}	0,036	0,128***	-0,169	0.049
	N_A	8	10	10	11

Table 2. Analysis of molecular variance (AMOVA) for Oenocarpus bataua partionning variation in 8 microsatellite loci by localities (3 populations ; n=90 inds .), by ecotypes (2 ecotypes ; n=90 inds.) and Intuto population by ecotypes (2 ecotypes ; n=30).

		d.f	Variance component	Percentage of variation	F_{ST}
By localities	Among populations	2	0,337	10,57	
	Within population	177	2,852	89,43	0,105***
By ecotypes	Among ecotypes	1	0,202	6,19	
	Within ecotypes	178	3,073	93,81	0,061***
Intuto by ecotypes	Among ecotypes	1	0,003	0,16	
	Within ecotypes	58	2,097	99,84	0,001

Genetic differentiation

The inference of population structure using the software STRUCTURE 2.1 (Pritchard *et al*. 2000) was not straightforward. The uppermost level of structuring was attributed to a *K*=2, and as second option to K=4 (Figure 2). For K=2, most individuals from Pantoja were well segregated from the rest of the samples with *q* values > 0.9 to their respective cluster plus few individuals from Intuto; meanwhile, Jenaro Herrera samples with *q* values > 0.8 and most of the Intuto samples with a wide range of *q* values were included in the second cluster (Figure 3). The results for a *K*=2 were in agreement with the F_{ST} values suggesting that Jenaro Herrera and most of Intuto samples were genetically closer to each other than those of Pantoja. For *K*=4, Pantoja formed again a discrete group of samples with *q* values > 0.8. Jenaro Herrera and Intuto samples, formerly clustered with *K*=2, were split into two overlapping clusters. Jenaro Herrera (*ca*. 50% inds. with *q* values > 0.8) and most Intuto individuals (*ca*. 50% inds. with *q* values > 0.7) appeared as two distinctive clusters. A fourth group was formed by several individuals from the locality of Jenaro Herrera, Intuto and a single individual from Pantoja. The genotypes from Pantoja were clearly segregated in both K=2 and K=4. A weak spatial structure was detected with K=4 since Pantoja and at lesser extent Jenaro Herrera and Intuto individuals were segregated in separate clusters (Figure 2). The lack of a genotypic structuring among *O. bataua* samples, particularly among Intuto individuals, was also observed in the UPGMA tree (Figure 4).

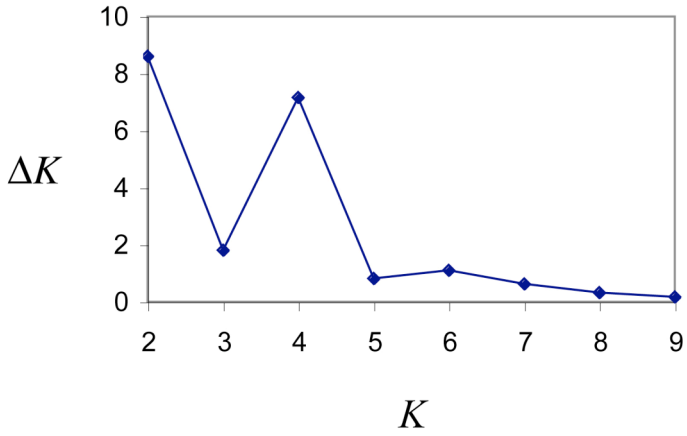


Figure 2. Delta K values for the number of inferred clusters (K)

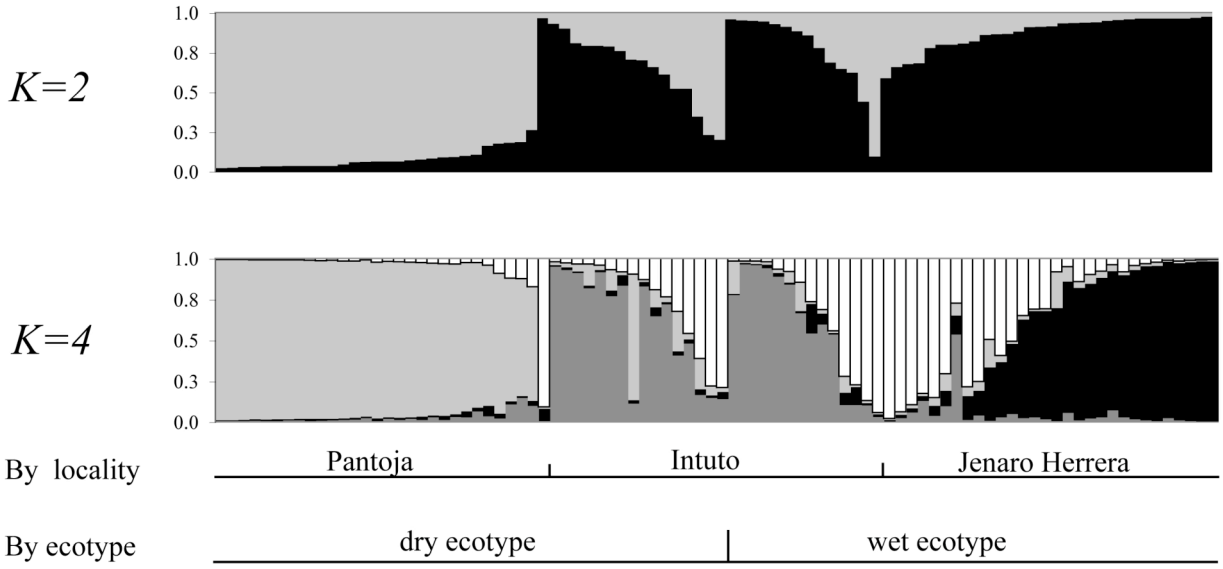


Figure 3. Estimated population structure with a K = 2 and K= 4. Each individual of *Oenocarpus bataua* is represented by a vertical bar, which is partitioned into K colored segments that represent the individual's estimated membership (*q* values) in K clusters. Populations are labeled below the figure by localities and ecotypes.

Discussion

Genetic structure

The values reported from AMOVA and F_{ST} for *Oenocarpus batua* populations reveals that molecular variability resides mostly within populations. Consequently, the genetic differentiation between populations was low (F_{ST} values) but statistically significant. The fact that most loci were not at Hardy-Weinberg Equilibrium when the three populations are pooled is also an indication of population differentiation. This pattern is quite similar to other out-crossed species from the tropics (Russell *et al*. 1999, Cardoso *et al*. 2000, Margis *et al*. 2002, Moretzsohn *et al*. 2002, Cavers *et al*. 2003, Salgueiro *et al*. 2004). However, in this study, within-population variation reached values extremely high, close to 90% of the total variation, despite the fact that distance between populations ranged from 180 to 400 km.

The weak among-populations genetic differentiation of *Oenocarpus bataua* is consistent with the regional setting in which the sampling has been realized, and in particular with the absence of physical barrier to gene flow. Isolation by distance and corresponding genetic differentiation may be limited due to:

(i) *O. bataua* is a predominately out-crossed species. This breeding strategy is related to higher gene flow and reduction of the genetic divergence (Hamrick & Godt 1996).

(ii) *O. bataua* is mainly pollinated by beetles (Borchsenius *et al.* 1998). Some of these pollinators would facilitate long-distance pollen movement which decrease geographical differentiation (Loveless & Hamrick 1984).

(iii) The fruits of *O. bataua* are largely consumed by frugivores (primates, birds, mammalians, rodents) as well opportunistic frugivores and omnivores (Peres 1994, Henry *et al*. 2000, Stevenson *et al*. 2000). Link & Di Fiore (2006) reported that primates like the "spider monkey" *Ateles belzebuth* are significant dispersers of *O. bataua* fruits, and that the average dispersal distance for seeds can reach up to 1281 m from their original source. The effect of the animal-mediated dispersal on genetic structure have not been studied in

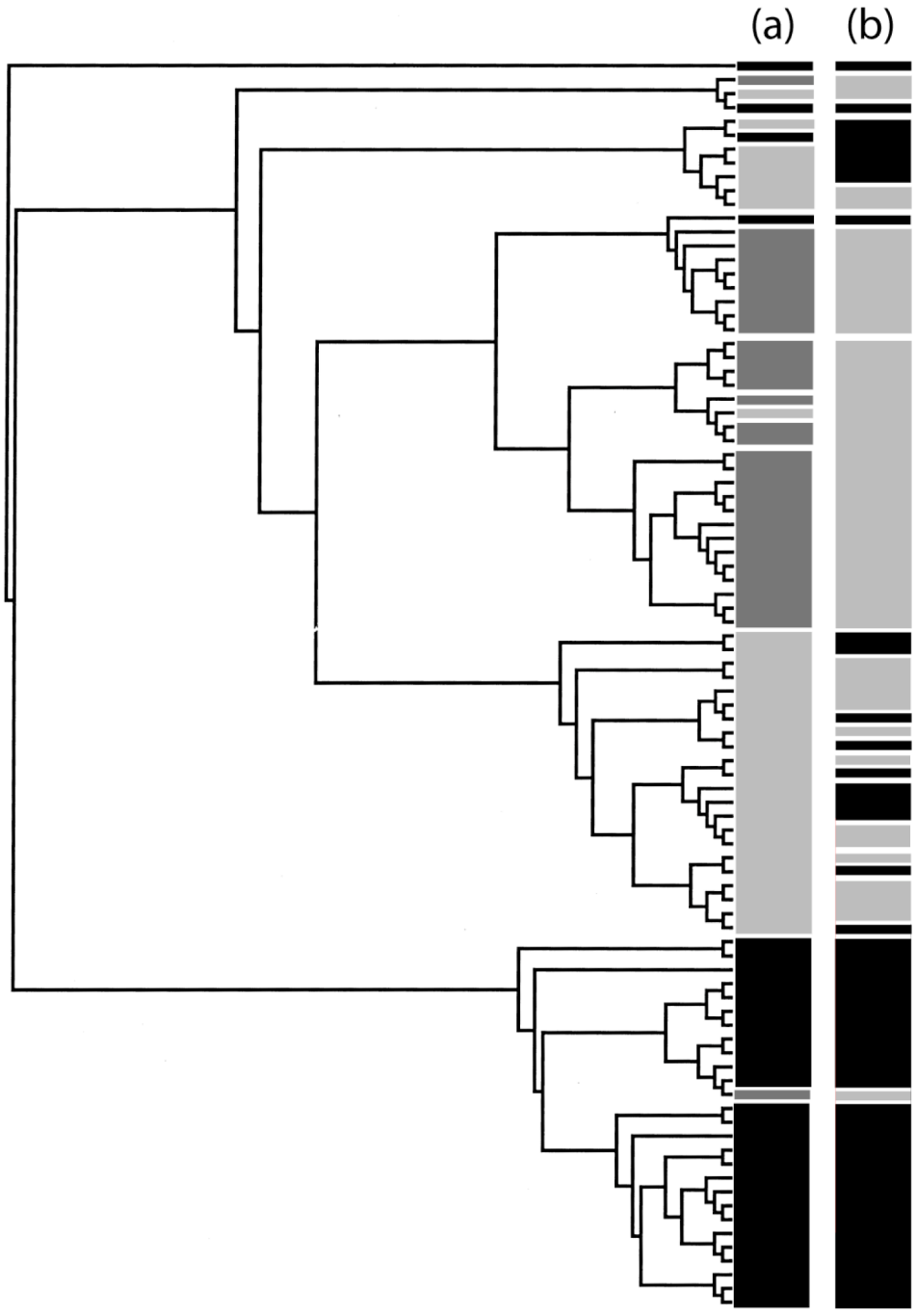


Figure 4. Distance tree UPGMA based on shared alleles distance. Horizontal bars represent the affiliation of each individual. The individuals were organized by populations (a) and ecotypes (b). By populations: black bars= Jenaro Herrera, dark gray bars= Pantoja, clear gray bars= Intuto. By ecotypes: black bars= wet ecotype, gray bars= dry ecotype.

O. bataua, however it is expected that frequent long-distance transport promotes homogeneity among populations (Loveless & Hamrick 1984).

(iv) Palynological and archaeological evidences reveal that Amerindians inhabitants have populated the Napo region 6-7000 years before present (Pitman 2000, Morcote-Rios & Bernal 2001). As humans are also consumers of the fruits, they may have acted as dispersers as well since a long time. Human-aided dispersal of *O. bataua* would promotes stochastic distributions that favor the genetic homogeneity over large spatial scales.

Population structure and ecological specialization

The weak among-population differentiation observed for *Oenocarpus bataua* populations was not an optimal condition to define a population sub-structure. However, Rosenberg *et al*. (2002) showed that even with poorly differentiated human populations, statistical analysis like STRUTURE can infers homogeneous groups of samples. In our study, the two models of clustering proposed by STRUCTURE software did not reveal any evident spatial or ecological structure. A spatial structuring of samples is weakly detected for a K=4 model, however, a fourth group of individuals (including some Jenaro Herrera and Intuto individuals) do not allow us to get a straightforward explanation of this genetic structure. In addition, no association was evidenced between the ecological preferences of individuals and the genetic structure. These results suggest that the variation in ecological preferences of *O. bataua* does not reflect the existence of specialized and genetically isolated ecotypes.

The assumption that a population is a discrete genetic unit (Ridley 2004) should be carefully applied to out-crossed species, widely and continuously distributed in the forest like *Oenocarpus bataua*. In this case, even with populations located 180-200 km apart, a weak evidence of a discrete genetic structure was detected. The wild populations studied would behave as functional and open units that allow significant amounts of gene flow among-populations, which are not constrained by physical barriers. This raises the question of the determinism of the clear ecocline of *Oenocarpus bataua* in the Napo region. The extremes of its geographical distribution (Pantoja and Jenaro Herrera) harbor populations specialized to opposite soil conditions (well-drained vs water-logged) while generalist populations grow in the intermediate area. Populations placed on the extreme ranges of its geographical distribution may correspond to real ecotypes. Theoretical and experimental

works suggest that a genotype is a integrated system adapted to an ecological niche (Snyder 1950, Van Valen 1975, Grant 1992). If we interpret the ecological niche as equivalent to an edaphic preference, so the connection genotype-ecology was not found for *O. bataua*. This evidence point out that ecological differentiation not necessarily promotes a genetic divergence.

Conclusions

Very little is known about the genetic structure of plant species from Western Amazon. However, this information reveals more about the natural history of this region and its dynamics. Further and broader studies encompassing new set of molecular markers are needed to precise the genetic structure of *Oenocarpus bataua* populations from this region. The shifts on the ecological preferences, the influences of fluvial systems as routes for the gene flow, the human influence are needed to be taken into account to understand the genetic structure of this palm species and the genetic dynamic in this region.

IV-*b* Variation in species composition, abundance and microhabitat preferences among western Amazonian *terra firme* palm communities

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The Palms
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Variation in species composition, abundance and microhabitat preferences among western Amazonian *terra firme* palm communities

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Western Amazonia harbours one of the richest palm floras of the Neotropics. About 121 palm species and 33 genera occur in this region. Approximately 40% of these species and three monotypic genera (*Aphandra*, *Itaya* and *Wendlandiella*) are restricted to western Amazonia. *Bactris* (23 spp.), *Geonoma* (20 spp.), *Attalea* (17 spp.), *Astrocaryum* (11 spp.) and *Oenocarpus* (7 spp.) are the most well-represented genera in the region. Palms, however, are not homogeneously distributed across western Amazonia. A major change in palm composition occurs between Yasuní (eastern Ecuador) and Iquitos (eastern Peru). Species that are very abundant on the unflooded forest of Yasuní, such as *Iriartea deltoidea* or *Prestoea shultzeana*, are replaced by *Socratea exorrhiza*, *Lepidocaryum tenue* var. *tenue* or *Iriartella stenocarpa* in the Iquitos–Pebas region. Moreover, the distribution ranges of the majority of eastern Ecuadorean palms reach the Iquitos region, but the converse is not observed. Censuses of palm communities along transects, studies of microhabitat preferences of *Oenocarpus bataua* and documentation of the distribution limit of *Astrocaryum* species in the intermediate zone provide new insights on the floristic change that is occurring. Modern ecological constraints and geological history during the Cenozoic may explain the observed variations. *Botanical Journal of the Linnean Society*, 2006, **151**, 127–140.

ADDITIONAL KEYWORDS: Arecaceae – biodiversity – biogeography – Iquitos arch – palm communities – western Amazonia.

INTRODUCTION

The purpose of this paper is to provide new insights into the distribution patterns of palms at regional spatial scales (~10^5 km^2) in western Amazonia. We will not test hypotheses about spatial distribution of tropical tree communities (stochastic vs. non-stochastic models), but rather discuss new findings from poorly studied areas lying between intensively studied sites (namely the Iquitos–Pebas and Yasuní regions) harbouring very distinct palm floras. During the last decade, many scientific approaches to the distribution patterns of palms have been applied in the northern part of western Amazonia (Pitman *et al*., 2002). In particular, palm floras from the westernmost part of the Amazon Basin, at the base of the Andean piedmont (eastern Ecuador), and Iquitos–Pebas region (north-east Peru) have largely been studied and recorded. Current assumptions and evidence on palm distribution in this region come largely from these two areas. However, the forest that covers the 500-km gap between eastern Ecuador and the Iquitos–Pebas region has been little studied. This intermediate region was our main area of interest. Inventories were

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made along the Tigre river (especially in the vicinity of the locality of Intuto, Peru) and the Napo river (from the Ecuadorean border to the river mouth) and compared with well-known sites in the region (Iquitos–Pebas, Jenaro Herrera and Yasuní, Fig. 1).

Western Amazonia

Western Amazonia or Upper Amazonia includes the tropical rain forest below 500 m a.s.l. on the eastern foothills of the Andean cordillera and adjacent lowlands lying eastward. Politically, it covers the Amazonian regions of Colombia, Peru, Ecuador, north-west Bolivia and western Brazil (the states of Amazonas and Acre). This region is well known for its overwhelming plant diversity (Gentry, 1988; Duivenvoorden & Lips, 1995; Pitman *et al*., 2002; Valencia *et al*., 2004). One hectare of forest on *terra firme* can harbour more than 300 species of trees ≥10 cm d.b.h. (Gentry, 1988; Valencia, Balslev & Paz y Miño, 1994; Pitman *et al*., 2002). Palm diversity is correspondingly high. Rich palm communities have been documented in the Iquitos–Pebas region (Kahn & Mejía, 1991; Vormisto, 2002; Vormisto *et al*., 2004a; Vormisto, Tuomisto & Oksanen, 2004b), Yasuní National Park (Svenning, 1999) and the Middle Caquetá basin, in eastern Colombia (Galeano, 1992).

To the north, the savanna formation and gallery forests in north-east Colombia (Departamento de Vichada) have been considered as a natural limit between the floristic regions of western Amazonia and Guayana. Duivenvoorden & Lips (1995) and Berry, Huber & Holst (1995) suggested that the westernmost outcrops of the Guayana shield (e.g. the low sandstone outcrops in Middle Caquetá river basin and the Serrania de la Macarena) could be a physical and floristic limit between these regions. To the west, the Andean slopes between 500 and 900 m a.s.l. (Balslev & Renner, 1989; Henderson, 1995) have been considered as the upper limit of most Amazonian plants. Some palm species widely distributed in the lowlands of western Amazonia, e.g. *Iriartea deltoidea*, *Oenocarpus bataua* and *Socratea exorrhiza*, reach the eastern slopes of the Andes up to 1000–1300 m a.s.l. Some Andean–Amazonian taxa have an even greater altitudinal range, such as *Bactris corossilla* (100–1400 m), *Wettinia maynensis* (100–1800 m), *Aiphanes weberbaueri* (100–2400 m) and *Chamaedorea pinnatifrons* (100–2500 m). In the south, there is no clear physical discontinuity, and several western Amazonian palms reach the north-western lowlands of Bolivia (Moraes *et al*., 1995). A progressive change from perhumid to seasonal climate towards the south-western part of Amazonia (Madre de Dios and Beni regions) is responsible for the impov-

Figure 1. Map of north-western South America showing the extent of the western Amazonia region (shaded area). The open squares indicate the study sites (Intuto, Jenaro Herrera and Yasuní). Other locality names mentioned in the text are indicated by solid squares.

erishment of the rainforest flora (Pitman *et al*., 2002) and eventually of the transition between western Amazonian forests and *cerrado* formation. The eastern limit of western Amazonia is very diffuse. The locality of Tefé in Amazonas State (64°42′W, Brazil; Ruokolainen & Vormisto, 2000) marks the eastern limit of distribution for many western Amazonian taxa (*Bactris sphaerocarpa*, *Geonoma laxiflora*), but some others, e.g. *Bactris balanophora*, *B. bidentula*, *B. bifida*, *B. concinna*, *B. killipii*, *B. macroacantha* and *B. riparia*, reach Manaus in central Amazonia (60°W, Fig. 1).

Geologically, western Amazonia or the Amazonian foreland basin includes the lowlands between the Andean slopes and the Iquitos arch (Räsänen *et al*., 1990). It is composed of at least four large intraforeland basins (Pastaza-Marañón, Ucayali, Madre de Dios-Beni, and Acre) separated by emerging ridges or arches (Vaupés arch, Serra do Moa arch, Fitzcarrald arch). The change of the western Amazonian landscape into intraforeland basins occurred mostly during the late Cenozoic (Räsänen *et al*., 1990). Although not very prominent in the landscape, these arches could have played a major role in the distribution patterns of the modern Amazon flora (Räsänen *et al*., 1990). The soils of western Amazonia consist of young and relatively fertile sediments deposited from the initiation of the Andean orogeny in the Miocene until now (Räsänen *et al*., 1992).

Palm communities in western Amazonia

Based on modern Neotropical palm monographs (Balick, 1986; Barfod, 1991; Kahn & Millán, 1992; Henderson, 1995, 2000; Henderson, Galeano & Bernal, 1995; Borchsenius & Bernal, 1996; Henderson & Galeano, 1996; Glassman, 1999), we have estimated the number of native palms in western Amazonia to be about 121 species and 33 genera (roughly two-thirds of the Amazon palms and one-fifth of the New World palms). Three monotypic genera and *c*. 60 palm species have distribution ranges centred in or restricted to western Amazonia. The western Amazonian palm flora is composed of different biogeographical elements, which account for its richness. (i) Typically Andean genera such as *Wettinia*, *Prestoea* and *Aiphanes* reach western Amazonian lowlands (Moraes *et al*., 1995). Palm species such as *Wettinia maynensis* and *Prestoea shultzeana* are very abundant in the lowland forests of eastern Ecuador and adjacent Peru; *Aiphanes deltoidea* and *Aiphanes weberbaueri* have been collected from north-east Peru (Departamento de Loreto); *Wettinia augusta*, *Wettinia drudei* and *Aiphanes ulei* occur in the lower Ucayali region (Peru). (ii) Species restricted to or centred in western Amazonian lowlands, including *Itaya amicorum*, *Chelyocarpus repens*, *C. ulei*, *Iriartella stenocarpa*, *Socratea salazarii*, *Oenocarpus circumtextus*, *O. makeru*, *O. simplex*, *Syagrus smithii*, *Ammandra dasyneura*, *Phytelephas macrocarpa*, *P. tenuicaulis*, *Astrocaryum chambira*, *Bactris bifida*, *B. killipii*, *Attalea insignis*, *Geonoma poeppigiana* and *G. camana*. (iii) Palms with a pan-Amazonian distribution, including the Guayanas region and Trinidad, e.g. *Euterpe precatoria* var. *precatoria*, *Mauritia flexuosa*, *Bactris simplicifrons*, *Mauritiella armata*, *Lepidocaryum tenue*, *Attalea maripa* and *Geonoma macrostachys*. (iv) A fourth element includes species with a distribution in tropical areas of South America (Amazon, Chocó, Caribbean region in northern Venezuela and Colombia, Brazilian Atlantic forest). In this group we find species such as *Oenocarpus bataua*, *Pholidostachys synanthera*, *Bactris hirta* var. *pectinata*, *Bactris brongniartii* and *Geonoma leptospadix* (v) Most of the remaining species have a Neotropical distribution extending well into Central America, such as *Socratea exorrhiza*, *Hyospathe elegans*, *Desmoncus orthacanthos*, *Geonoma deversa*, *G. stricta*, *Chamaedorea pinnatifrons*, *Iriartea deltoidea*, *Oenocarpus mapora*, *Attalea butyracea* and *Bactris maraja*. *Dictyocarym ptarianum* is a rare example of a Guayanan element with infrequent occurrence in western Amazonia.

The highest local palm richness was reported from the Iquitos region (north-east Peru) with more than 65 species and varieties from different habitats (Henderson, 1995; Vasquéz-Martínez, 1997). Vormisto (2002) recorded 58 species and varieties in 3.6 ha on *terra firme* forest of the Iquitos–Pebas region. Kahn & Mejía (1991) recorded 29 species and varieties in 0.71 ha and 34 species in 0.5 ha in two *terra firme* forests of the lower Ucayali river. High palm richness was also found in other western Amazonian localities. In Yasuní National Park in Ecuadorian Amazonia, 54 palm species and varieties from different habitat were recorded (R. Montúfar, unpubl. data), and 30 species and varieties within 4.7 ha of *terra firme* forest (Svenning, 1999). Galeano (1992) recorded 64 palm species and 26 genera in the Araracuara region, eastern Colombia.

Western Amazonia and the nearby eastern and western Andes constitute the centre of diversity of the Iriarteeae (Spruce, 1871; Malagón & Bernal, 2002). All Iriarteeae genera (*Iriartea*, *Wettinia*, *Socratea*, *Iriartella* and *Dictyocaryum*) are present in western Amazonia. Although not as species rich as other palm groups (no more than three species per genus, nine in total), they are a conspicuous element as they usually occur in high density. *Iriartea deltoidea* and, to a lesser extent, *Socratea exorrhiza* are abundant palms on the lowland tropical forests close to the Andes. *Iriartella stenocarpa* and *Wettinia drudei* are dominant

components of the understorey in some forests further to the east.

Bactris (Arecoideae, Cocoseae, Bactridinae) and *Geonoma* (Arecoideae, Geonomateae) are the most well-represented genera in western Amazonia with 23 and 20 species, respectively. Both genera include small acaulescent or caulescent understorey palms (< 3 m tall). *Astrocaryum* (Arecoideae, Cocoseae, Bactridinae) includes 12 species in western Amazonia, according to Kahn & Millán (1992). *Attalea s.l.* (i.e. including *Scheelea*, *Attalea*, *Orbignya* and *Maximiliana*) is represented by 17 species, according to Glassman (1999). *Oenocarpus* (Arecoideae, Euterpeae) includes some species with economic value. Seven species within this genus occur in western Amazonia; three of them (*O. makeru*, *O. circumtextus* and *O. simplex*) have been found only in a small area in eastern Colombia and adjacent Brazil (Bernal, Galeano & Henderson, 1991; Henderson, 1995). Three monotypic endemic genera occur in western Amazonia: *Itaya* (closely related to *Chelyocarpus*), *Wendlandiella* (closely related to *Synechanthus* and *Chamaedorea*) and *Aphandra* (allied to *Phytelephas* and *Ammandra*).

MATERIAL AND METHODS

In order to document changes in floristic composition and ecology of species in palm communities of western Amazonia (the region lying between the Andean Piedmont of north-east Ecuador and the lowlands of north-east Peru, Fig. 1), we established three study areas.

(I) Yasuní, located within the Yasuní National Park, in eastern Ecuador (1°S, 76°W). The geomorphology is a system of low hills, below 400 m elevation, at the base of the Andean Piedmont, and drained by the upper Napo River. This site and its palm communities have been extensively studied and described previously (Svenning, 1999; Pitman *et al*., 2002; Valencia *et al*., 2004; Vormisto *et al*., 2004a).

(II) Intuto (3°27′S, 74°45′W), located on the middle course of the Tigre River, in the Loreto region of Peru (170 km WNW from Iquitos and 360 km SSE of Yasuní). The geomorphology is a low hill system (below 300 m elevation), with steep slopes and narrow valleys, at the western margin of the Iquitos Arch. This site has never been studied previously and is located in the poorly known intermediate zone of the region studied.

(III) Jenaro Herrera (4°58′S, 73°45′W), located on the right bank of the lower Ucayali River, Loreto region, Peru. The geomorphology comprises flat areas close to the Ucayali River, including alluvial terraces with various degrees of hydromorphy and white sand deposits, and also low hills, becoming progressively higher and steeper to the east, forming the south-western limit of the Iquitos Arch. Two palm communities have been previously studied at this site (Kahn & Mejía, 1991).

Fourteen 500 × 5-m transects were established on *terra firme* forest (six in Intuto and eight in Jenaro Herrera). In these transects, all palm individuals were recorded and identified to species and variety (when possible). In Yasuní region, twelve 500 × 5-m transects were established on *terra firme*. All individuals of *Oenocarpus bataua* (including seedlings) were recorded among these transects (Montúfar, 1999). Corresponding herbarium collections were deposited at QCA and USM (Holmgren, Holmgren & Barnett, 1990). Data for the other species at Yasuní were taken from Vormisto *et al*. (2004a).

The topography (angle of inclination) along the transects was recorded each 5 m using a clinometer. From the former data, topographic profiles were constructed for each transect and the average height with respect to the altitudinal range of the transect was calculated every 5 m. Drainage categories were taken every 5 m using two qualitative drainage classes: (1) well drained and (2) waterlogged. These data were used to test correlations between topography and the spatial distribution of *Oenocarpus bataua* (microhabitat preferences) in all three study sites.

We classified each species in a biogeographical unit, in order to determine the relative importance of various biogeographical components in each locality. The units are: Andean (includes species occurring above 800 m elevation in the Andes and extending downward to western Amazonia); western Amazonian endemic (below 800 m elevation) and western Amazonian centred (extending to central Amazonia); pan-Amazonian (including the Guayanas and Trinidad), following the delimitation of the Amazon region of Henderson (1995); South American (with a trans-Andean distribution or bipolar Amazonian–Brazilian Atlantic forest distribution); and Neotropical (including species occurring from central America to South America). Additional distribution data of western Amazonian palm species were taken from the above-mentioned literature, to help interpretation of floristic changes between sites.

Finally, the exact distribution limits of two species representative of the floristic changes occurring in the region studied, *Astrocaryum urostachys* and *Astrocaryum macrocalyx*, were documented along the Napo, Marañón and Tigre rivers. Characters used to differentiate these two closely related species were taken from Kahn & Millán (1992) and include habit (caespitose in *A. urostachys*, solitary in *A. macrocalyx*) and female flower structure (calyx sparsely setose with wrinkled limb in *A. urostachys*, glabrous with straight, constricted limb in *A. macrocalyx*).

Nomenclature of palm species follows Govaerts & Dransfield (2005) except for four species. We use

Attalea ferruginea instead of *Attalea racemosa* because the latter name is based on a type lacking staminate flowers, which are critical for indentification in *Attalea*. Moreover, *A. racemosa* has been transferred to *Orbignya* by Drude, adding even more confusion, as the taxon considered in our study has flowers of *Attalea s.s.* and not of the *Orbignya* type. We also use *Attalea polysticha* instead of *A. microcarpa* because the type of the latter also lacks staminate flowers and because the concept of *A. microcarpa* used by Henderson (1995) includes *A. sagotii* from the Guyanas, which shows significant differences from the western Amazon taxon we have sampled. Our taxon corresponds unambiguously to *A. polysticha*, the type of which is from Iquitos. Our arguments on the use of *Attalea* names are based on Glassman (1999). We also maintain *Aiphanes schultzeana* as distinct from *A. ulei*, in order to distinguish the medium-sized, trunked form abundant in the Andean Piemont (*A. schultzeana*) from the diminutive acaulescent form encountered in north-east Peru (*A. ulei*, the neotype of which is from one of the studied sites, Jenaro Herrera). Although these two distinct morphotypes are well documented (Borchsenius, Borgtoft & Balsev, 1998), this is not reflected in the current nomenclature in which *A. schultzeana* is considered to be a synomym of *A. ulei*. Finally, we use *Ammandra dasyneura* (western Amazon endemic) instead of a broad concept of *A. decasperma* as a conservative approach, because we consider that morphological variation within the genus is still insufficiently documented (Bernal, Ramírez & Morales, 2001).

Data analysis

The structure and composition of palm communities were evaluated by computing the relative abundance of each species. Species diversity among study sites (Intuto and Jenaro Herrera) was calculated using Shannon's Index (H'; Shannon, 1948). The ten most abundant species in each site were compared using histograms. Proportions of biogeographical components in each site were computed on the basis of the number of species and number of individuals belonging to each component.

We used two statistical approaches to explore the relationship between the spatial distribution of *Oenocarpus bataua* and topography. We first used the Mantel test (Legendre & Legendre, 1998), with 129 sampling units of 25×5 m (41 for Intuto, 33 for Jenaro Herrera and 55 for Yasuní) selected from the 26 transects of 500×5 m. The sampling units were placed on two well-defined topographic positions: valley bottoms and hill tops. The distance among sampling units varied between 50 and 100 m within a transect to avoid spatial autocorrelation (Legendre & Legendre, 1998). The total number of individuals of *O. bataua* taller than 1 m was recorded for each sampling unit. Topography-drainage was composed of three variables coded for each sampling unit: (1) average angle of inclination, (2) average height with respect to the altitudinal range of the transect and (3) drainage coded as a binary variable (well-drained sites vs. waterlogged sites).

The similarity matrix based on abundance of *O. bataua* individuals taller than 1 m was computed using the Steinhaus coefficient (Legendre & Vaudor, 1991). Topographic similarities (angle of inclination, average height and drainage) were calculated with Euclidean distance. The Mantel test calculates a linear correlation coefficient (r) between similarity matrices. We used a standardized form of this test, in which the values of the correlation coefficient r vary between -1 and $+1$. The statistical significance (P) of Mantel r-values was calculated with 999 permutations. Statistical analyses described above were computed with R-package software (Legendre & Vaudor, 1991).

In a second analysis, a logistic regression was implemented to analyse the degree of association between the distribution of subadult and adult trees of *O. bataua* (more than 5 m high) and drainage (well-drained sites vs. waterlogged sites). Individuals more than 5 m high were recorded for the 129 sampling units described above, and the drainage was coded as a binary variable as in the previous analysis. Logistic regression was computed with StatView (SAS Company, 1998).

RESULTS

Composition of palm communities

We recorded 10 019 individuals and 52 palm species and varieties from Jenaro Herrera transects, and 8628 individuals and 41 species and varieties from Intuto (Table 1). The ten most abundant taxa in Jenaro Herrera accounted for 80.9% of the individuals sampled, and the other 42 species represented the remaining 19.1%. In Intuto, the ten most abundant species and varieties accounted for 91%, and the other 31 species and varieties represented 9% of the total palm community (Fig. 2). In both localities we found an oligarchic dominance. *Lepidocaryum tenue* var. *tenue* and *Oenocarpus bataua* accounted for 58% of the individuals sampled in Jenaro Herrera, and *Iriartella stenocarpa* and *O. bataua* represented 50% of all individuals registered in Intuto (Table 1). Excluding a few unidentified species (mostly *Bactris*), 30 species and varieties were shared by both localities; 20 and ten species and varieties were restricted to Jenaro Herrera and Intuto, respectively. A list of palm species

Table 1. Palm species and varieties recorded in Intuto and Jenaro Herrera. Records for Iquitos–Pebas and Yasuní were taken from Vormisto *et al*. (2004a). BE = biogeographical elements: An = Andean, WA = western Amazonian, Am = Pan-Amazonian, SA = South American, N = Neotropical. Study sites: I, Intuto (number of species recorded); JH, Jenaro Herrera; IP, Iquitos–Pebas; Y, Yasuní. *N* = number of individuals, % = relative abundance. Species and varieties are arranged in alphabetical order

		JH (52spp.)		I (41spp.)		Y (36spp.)		IP (54spp.)	
Palm species and varieties	BE	*N*	%	*N*	%	*N*	%	*N*	%
Aiphanes schultzeana Burret	An					109	0.87		
Aiphanes ulei (Dammer) Burret	WA	1	0.01					6	0.05
Aiphanes weberbaueri Burret	An			7	0.08				
Ammandra dasyneura (Burret) Barfod	WA					281	2.24		
Aphandra natalia (Balslev & Henderson) Barfod	WA					3	0.02		
Astrocaryum chambira Burret	WA	31	0.31	29	0.34	279	2.23	93	0.76
Astrocaryum javarense (Trail) Drude	WA	329	3.28						
Astrocaryum murumuru Mart. (*A. macrocalyx* Burret + *A. javarense* (Trail) Drude)	WA							1 330	10.86
Astrocaryum urostachys Burret	An					151	1.21		
Attalea sp1	–	17	0.17						
Attalea ferruginea Burret	An			129	1.50				
Attalea insignis (Mart.) Drude	WA	146	1.46	30	0.35	1	0.01		
Attalea maripa (Aubl.) Mart.	Am	1	0.01			237	1.89	124	1.01
Attalea plowmanii (Glassman) Zona	WA							542	4.42
Attalea polysticha (Burret) Wess.Boer	Am	110	1.10						
Bactris acanthocarpa Mart. var. *exscapa* Barb.Rodr.	Am	103	1.03	52	0.60			17	0.14
Bactris sp1	–	14	0.14						
Bactris sp2* (Vormisto *et al*. 2004a)	–							38	0.31
Bactris sp3	–	2	0.02						
Bactris sp4	–			20	0.23				
Bactris aff. major var. *infesta* (Mart.) Drude	Am							24	0.20
Bactris bifida Mart.	WA	76	0.76					52	0.42
Bactris concinna Mart.	WA			5	0.06	26	0.21		
Bactris corossilla H.Karst.	SA	40	0.40			43	0.34		
Bactris fissifrons Mart.	WA							89	0.73
Bactris halmoorei A.J.Hend.	WA			12	0.14			32	0.26
Bactris hirta Mart. var. *hirta*	WA			12	0.14			35	0.29
Bactris hirta Mart. var. *lakoi* (Burret) A.J.Hend.	WA	26	0.26	41	0.48			48	0.39
Bactris hirta Mart. var. *pectinata* (Mart.) Govaerts	SA	12	0.12	92	1.07				
Bactris killipii Burret	WA	57	0.57	3	0.03			5	0.04
Bactris macroacantha Mart.	Am							51	0.42
Bactris maraja Mart. var. *chaetospatha* (Mart.) A.J.Hend.	WA	133	1.33						
Bactris maraja Mart. var. *juruensis* (Trail) A.J.Hend.	WA	10	0.10			27	0.22		
Bactris maraja Mart. var. *maraja*	N	6	0.06	19	0.22	13	0.10	58	0.47
Bactris maraja Mart. var. *trichospatha* (Trail) A.J.Hend.	Am							17	0.14
Bactris schultesii (L.H.Bailey) Glassman	WA	14	0.14	2	0.02	10	0.08	13	0.11
Bactris simplicifrons Mart.	Am	106	1.06	51	0.59	1	0.01	69	0.56
Bactris sphaerocarpa Trail	WA	95	0.95					23	0.19
Chamaedorea pauciflora Mart.	An	91	0.91	3	0.03	20	0.16	17	0.14
Chamaedorea pinnatifrons (Jacq.) Oerst.	N	7	0.07	1	0.01	141	1.13	1	0.01
Chelyocarpus repens Kahn & Mejia	WA	116	1.16					68	0.56
Desmoncus giganteus A.J.Hend.	WA	3	0.03	1	0.01	3	0.02	5	0.04
Desmoncus mitis Mart. var. *mitis*	WA			5	0.06	9	0.07	2	0.02
Desmoncus mitis Mart. var. *tenerrimus* (Mart. ex Drude) A.J.Hend.	WA							16	0.13
Desmoncus orthacanthos Mart.	N	1	0.01	4	0.05	3	0.02		

Table 1. *Continued*

Palm species and varieties	BE	JH (52spp.)		I (41spp.)		Y (36spp.)		IP (54spp.)	
		N	%	*N*	%	*N*	%	*N*	%
Desmoncus polyacanthos Mart.	Am	3	0.03	2	0.02			15	0.12
Euterpe precatoria Mart.	Am	347	3.46	30	0.35	246	1.96	399	3.26
Geonoma sp1	–			80	0.93				
Geonoma arundinacea Mart.	WA							41	0.33
Geonoma aspidiifolia Spruce	Am					92	0.73		
Geonoma atrovirens Borchs. & Balslev	WA			32	0.37				
Geonoma brongniartii Mart.	WA	3	0.03	1	0.01	144	1.15	95	0.78
Geonoma camana Trail	WA	4	0.04					82	0.67
Geonoma deversa (Poit.) Kunth	N	12	0.12	16	0.19			181	1.48
Geonoma leptospadix Trail	Am	2	0.02	1	0.01			2	0.02
Geonoma macrostachys Mart. var. *acaulis* (Mart.) Skov	Am	346	3.45	474	5.49			142	1.16
Geonoma macrostachys Mart. var. *macrostachys*	WA	20	0.20			1 915	15.29	1 098	8.96
Geonoma maxima (Poit.) Kunth var. *chelidonura* (Spruce) A.J.Hend.	Am	162	1.62	193	2.24			286	2.33
Geonoma maxima (Poit.) Kunth var. *maxima*	Am					105	0.84	7	0.06
Geonoma poeppigiana Mart.	WA	60	0.60					106	0.87
Geonoma stricta (Poit.) Kunth var. *piscicauda* (Dammer) A.J.Hend.	WA					33	0.26	793	6.47
Geonoma stricta (Poit.) Kunth var. *stricta*	Am	63	0.63	59	0.68	140	1.12	23	0.19
Geonoma stricta (Poit.) Kunth var. *trailii* (Burret) A.J.Hend.	Am	66	0.66	409	4.74	47	0.38	58	0.47
Geonoma triglochin Burret	An			2	0.02	15	0.12		
Hyospathe elegans Mart.	N	258	2.58	129	1.50	94	0.75	291	2.38
Iriartea deltoidea Ruiz & Pav.	N	174	1.74			1 999	15.96	793	6.47
Iriartella stenocarpa Burret	WA	182	1.82	2494	28.91			370	3.02
Lepidocaryum tenue Mart. var. *tenue*	WA	3 426	34.20	1653	19.16			2 160	17.63
Mauritia flexuosa L.f.	Am	191	1.91						
Oenocarpus balickii F.Kahn	WA	50	0.50					38	0.31
Oenocarpus bataua Mart.	SA	2 349	23.45	1851	21.45	3 137	25.04	878	7.17
Oenocarpus mapora H.Karst.	N	3	0.03	22	0.25	6	0.05	93	0.76
Pholidostachys synanthera (Mart.) H.E. Moore	SA	142	1.42						
Phytelephas macrocarpa Ruiz & Pav.	WA	15	0.15					120	0.98
Phytelephas tenuicaulis (Barfod) A.J.Hend.	WA					390	3.11	19	0.16
Prestoea schultzeana (Burret) H.E. Moore	An					2 536	20.24	18	0.15
Socratea exorrhiza (Mart.) H.Wendl.	N	498	4.97	31	0.36	74	0.59	1 238	10.11
Socratea salazarii H.E. Moore	WA	28	0.28	155	1.80				
Syagrus sancona H.Karst.	SA					1	0.01		
Syagrus smithii (H.E. Moore) Glassman	WA			161	1.87				
Wendlandiella gracilis Dammer var. *polyclada* (Burret) A.J.Hend.	WA							129	1.05
Wettinia augusta Poepp. & Endl.	WA	51	0.51						
Wettinia drudei (O.F.Cook & Doyle) A.J.Hend.	WA	17	0.17	315	3.65				
Wettinia maynensis Spruce	An					197	1.57	1	0.01
Total		10 019	100	8628	100	12 528	100	12 251	100

found in both localities is provided in Table 1. Shannon's Index of species diversity for these sites and Yasuni and Iquitos–Pebas (taken from Vormisto *et al*., 2004a) are given in Table 2.

The Andean element was more conspicuous in areas closer to the Andes like Yasuní where 17% of the species and 24% of the individuals sampled corresponded to this biogeographical group of palms. Eastward, the western Amazon component become predominant (Iquitos–Pebas: spp. 51.8%, ind. 60.4%; Jenaro Herrera: spp. 46.1%, ind. 48.8%). The species number as well as the number of individuals of pan-Amazonian

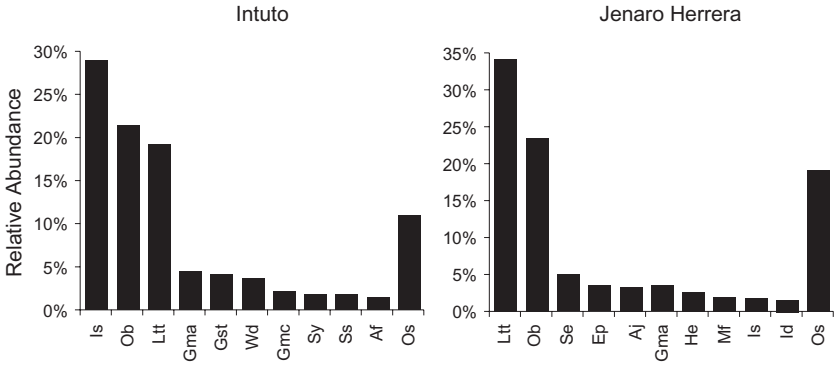

Figure 2. Relative abundance of the ten most abundant species and varieties in Intuto and Jenaro Herrera. Species names: Af, *Attalea ferruginea*; Aj, *Astrocaryum javarense*; Ep, *Euterpe precatoria*; He, *Hyospathe elegans*; Id, *Iriartea deltoidea*; Is, *Iriartella stenocarpa*; Gma, *Geonoma macrostachys* var. *acaulis*; Gmc, *Geonoma maxima* var. *chelidonura*; Gst, *Geonoma stricta* var. *trailii*; Ltt, *Lepidocaryum tenue* var. *tenue*; Mf, *Mauritia flexuosa*; Ob, *Oenocarpus bataua*; Se, *Socratea exorrhiza*; Ss, *Socratea salazarii*; Sy, *Syagrus smithii*; Wd, *Wettinia drudei*; Os, *other species*.

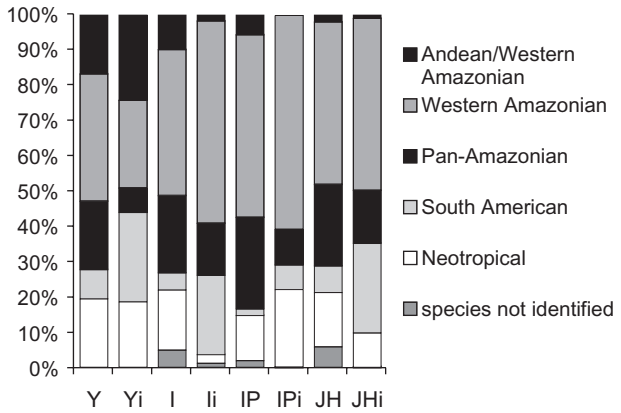

Figure 3. Geographical distribution patterns of the palm species and varieties recorded in Intuto (I, number of species; Ii, number of individuals) and Jenaro Herrera (JH, JHi). Data for Iquitos–Pebas (IP, IPi) and Yasuní (Y, Yi) were taken from Vormisto *et al*. (2004a).

and Neotropical palms remained constant in all areas. Widely distributed South American palms were represented by few species, among which is one of the most abundant recorded, *Oenocarpus bataua* (Fig. 3).

Association of *Oenocarpus bataua* with topography

The correlation between the spatial distribution of *Oenocarpus bataua* and topography–drainage was moderate among the sampling units of Jenaro Herrera and Yasuní, but highly significant in the latter locality (Table 3). In Jenaro Herrera, individuals of *O. bataua* are preferentially associated with waterlogged soils, whereas in Yasuní they clearly favour well-drained soils. By contrast, no correlation was obtained between the spatial distribution of *O. bataua* and topography–drainage in the sampling units of Intuto (Table 3).

The results of logistic regression showed that the distribution of *O. bataua* was related to drainage conditions in Yasuní ($r^2 = 0.215$, $P = 0.0001$) and Jenaro

Table 2. Shannon's Index of species diversity (H'), number of species and varieties (S), number of individuals (No. ind.), sampled area (Area) and the geographical distance between transects (GDT). Data for Iquitos–Pebas and Yasuní were taken from Vormisto *et al*. (2004a)

	H'	S	No. ind.	Area (ha)	GDT (km)
Intuto	2.20	41	8 628	1.5	0.5–30
Iquitos–Pebas	2.93	54	12 251	2.75	0.5–170
Jenaro Herrera	2.44	52	10 019	2.25	0.5–19
Yasuní	2.26	36	12 528	2.5	1–18

Table 3. Coefficients of correlation (Mantel test) between floristic similarity matrices based on the abundance of *Oenocarpus bataua* and topography–drainage matrices. Statistical significance (P-values) was calculated with 999 permutations. $^{***}P < 0.001$, $^{NS}P > 0.05$

	Topography–drainage	
Study area	r	P
Intuto	0.077	0.157^{NS}
Jenaro Herrera	0.148	0.061^{NS}
Yasuní	0.149	0.001^{***}

Hererra ($r^2 = 0.213$, $P = 0.002$). No association was detected in Intuto ($r^2 = 0.004$, $P = 0.65$).

Distribution limit of *Astrocaryum urostachys* and *Astrocaryum macrocalyx*

We found two points of contact between these species, both located on the edge of the Iquitos Arch. The first contact zone was located on the Napo River (Pintaud, 2005), at the north-eastern limit of the Iquitos Arch (2°20′S, 73°48′W). The second contact zone was located on the western margin of the Iquitos Arch, near the confluence of the Rio Tigre and Rio Marañón rivers (4°28′S, 74°04′W). All herbarium records of both species are mapped on Figure 4.

DISCUSSION

Floristic changes in western Amazonia

In recent years, western Amazonia has been subject to many studies focused on the spatial distribution patterns of plants. Recent works have explored the spatial distribution of trees (Pitman *et al*., 2000, 2001), palms (Ruokolainen & Vormisto, 2000; Vormisto *et al*., 2004a,b) and other plant taxa (Tuomisto, Ruokolainen & Yli-Halla, 2003). These studies have suggested that plant communities tend to be (i) uniform over large areas and dominated by a limited set of ecologically superior species (Pitman *et al*., 2001) or (ii) heterogeneous and environmentally determined (Tuomisto *et al*., 2003). Moreover, Pitman *et al*. (2000) suggested that palm communities are largely predictable over very large areas of the Amazon Basin. Ruokolainen & Vormisto (2000) suggested that arborescent palms tend to be widespread and habitat generalists. Vormisto *et al*. (2004a) discussed the regional patterns of palms between eastern Ecuador and the Iquitos–Pebas region, and suggested that palm communities are more influenced by dispersal across broader spatial scales than by environmental heterogeneity.

Western Amazonia is a spatially heterogeneous region. This physical heterogeneity is a consequence of modern processes (e.g. fluvial dynamics, climatic gradients) and historical events (e.g. tectonics, Pleistocene climatic changes). Given these spatial and historical variations, are palm species homogeneously distributed and palm communities largely predictable at a regional scale? Our field observations indicate sharp floristic changes across short distances in the eastern Ecuador – north-east Peru region. These floristic changes are especially marked at the community level not only because of species distribution limits within the region, but also because of changes in ecology of widespread species across the region. Some examples of such phenomena are given below.

(i) The distribution ranges of most eastern Ecuadorean palms reach Iquitos, but the converse is not observed. According to available literature (Kahn & de Granville, 1992; Vasquéz-Martínez, 1997; Borchsenius *et al*., 1998; Svenning, 1999; Vormisto *et al*., 2004a), and our data, almost all eastern Ecuador palms occur

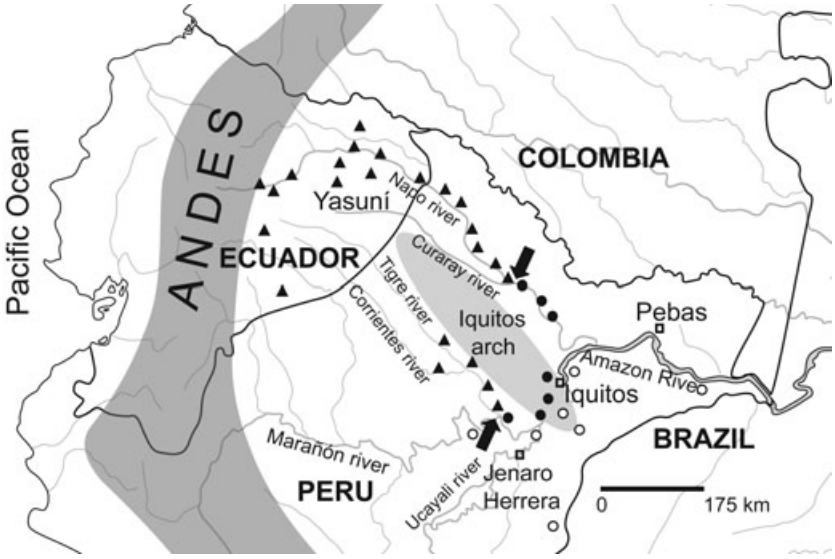

Figure 4. Spatial distribution of *Astrocaryum urostachys* (solid triangles), *A. macrocalyx* (solid circles) and *A. javarense* (open circles) in western Amazonia. Records of *A. urostachys* for Ecuadorean Amazonia were taken from herbarium data. The solid arrows indicate the contact zone between *A. urostachys* and A. *macrocalyx.* Light grey area represents the Iquitos Arch.

in the region of Iquitos. Among the 36 species and varieties recorded from Yasuní (Vormisto *et al*., 2004a), only five species were not reported from the Iquitos–Pebas region (Vormisto *et al*., 2004a) and Jenaro Herrera: *Ammandra dasyneura*, *Aphandra natalia*, *Geonoma aspidiifolia*, *G. triglochin* and *Syagrus sancona* (Table 1). *Ammandra dasyneura* has a small distribution area in eastern Ecuador and adjacent Colombia, *Geonoma triglochin* is restricted to the Andean Piedmont and *Syagrus sancona* has a highly disjunct distribution in western Amazonia, with a vast gap encompassing the Iquitos region. *Aphandra natalia* seems similarly absent from the Iquitos–Pebas region, but occurs next to it to the north-west and south-east. *Geonoma aspidiifolia* has a relatively large distribution, from north-west Amazonia to the Guayana highlands, but its pattern of occurrence within this area is poorly understood.

Astrocaryum urostachys, considered as endemic to eastern Ecuador (Borchsenius *et al*., 1998), has been recorded along most of the course of the Napo and Tigre rivers, reaching the Marañón River (Figure 4). Species segregated from the *Geonoma macrostachys* complex (*G. polyandra*, *G. supracostata*, *G. atrovirens*) were described from eastern Ecuador only (Skov, 1994; Borchsenius, Balslev & Svenning, 2001), but are likely to have a wider distribution range. Identification of these rather cryptic species requires optimal material, including staminate flowers, which makes them difficult to track among existing herbarium collections. Additional field work in north-east Peru will probably unravel populations of all these species. In fact, we encountered a large population of *Geonoma atrovirens* in Intuto (Table 1).

In contrast to Ecuadorean palms, many north-east Peruvian palms do not reach the Andean piedmont in the direction of Ecuador. Among the 54 species and varieties recorded from the Iquitos–Pebas region, 21 have never been reported from eastern Ecuador (Vormisto *et al*., 2004a), and among 52 species and varieties recorded from Jenaro Herrera, 27 were not recorded in Yasuní (excluding unidentified morphotypes, Table 1). Typical north-east Peruvian palms such as *Lepidocaryum tenue* var. *tenue*, *Chelyocarpus repens*, *Iriartella stenocarpa*, *Wettinia augusta*, *Wendlandiella gracilis*, *Bactris killipii*, *Bactris macroacantha* and *Attalea polysticha* have not been collected from eastern Ecuador. Around Intuto, 215 km from the Ecuadorean border, palm communities are still characterized by species typical of the Iquitos region, including *Lepidocaryum tenue* var. *tenue*, *Socratea salazarii*, *Iriartella stenocarpa*, *Wettinia drudei*, *Attalea ferruginea* and *Chelyocarpus repens*, but also comprise elements abundant in the Yasuní region such as *Geonoma triglochin*, *Syagrus smithii*, *Geonoma atrovirens* and *Iriartea deltoidea*, all of which become rarer or absent around Iquitos.

The floristic differences between eastern Ecuador and the Iquitos–Pebas region reflect the predominance of some biogeographical components in each region. Yasuní, being at the base of the Andean Piedmont, logically has a high proportion of Andean elements (six species, 16.6%) in contrast with the 9.7, 5.5 and 1.9% for Intuto, Iquitos–Pebas and Jenaro Herrera, respectively. By contrast, 52% of the palm flora of the Iquitos–Pebas region is composed of species with a geographical distribution centred in or restricted to western Amazonia. Widely distributed elements (Amazonian, South American and Neotropical) are evenly distributed in western Amazonia (Fig. 3).

(ii) Some palm species tend to be highly dominant (and sometimes oligarchic) in one region and rare or at low density in another. Prestoea shultzeana is the most abundant understorey palm on *terra firme* in Yasuní (eastern Ecuador). However, this species is either absent or present at very low density in Jenaro Herrera and Iquitos–Pebas, respectively (Vormisto *et al*., 2004a). *Iriartea deltoidea* is the most abundant arborescent palm species on *terra firme* in Yasuní, but in north-east Peru it becomes patchily distributed or rare over large areas. Similar trends can be detected in other palms such as *Phytelephas tenuicaulis*, *Astrocaryum chambira*, *Wettinia maynensis*, *Chamaedorea pinnatifrons*, *Aiphanes schultzeana* and *Geonoma macrostachys*. By contrast, palm species such as *Hyospathe elegans* or *Socratea exorrhiza* occur at high density in north-east Peru but at moderate density in eastern Ecuador (Fig. 5).

(iii) Ecological shifts in the local distribution patterns of palm species. A good example of such a phenomenon is the distribution of *Oenocarpus bataua*. This species grows on steep slopes in the Andes, up to 1000 m elevation or more. Descending the Andes, it maintains its edaphic preferences to unflooded (*terra firme*) forest, becoming an ecological indicator of well-drained conditions in eastern Ecuador (Balslev *et al*., 1987; Svenning, 1999; Vormisto *et al*., 2004a). Only uncommon communities of *O. bataua* – *Mauritiella armata* on swamps formed by the accumulation of organic matter have been reported in the south part of Yasuní. By contrast, the edaphic preferences of *O. bataua* for waterlogged soils has been reported in many localities of central Amazonia (Kahn & de Granville, 1992). Our data suggest that the shift in edaphic preferences of this species occurs within the region studied. Mantel test results and logistic regression indicated that changes in the abundance of *O. bataua* are related to topography and drainage

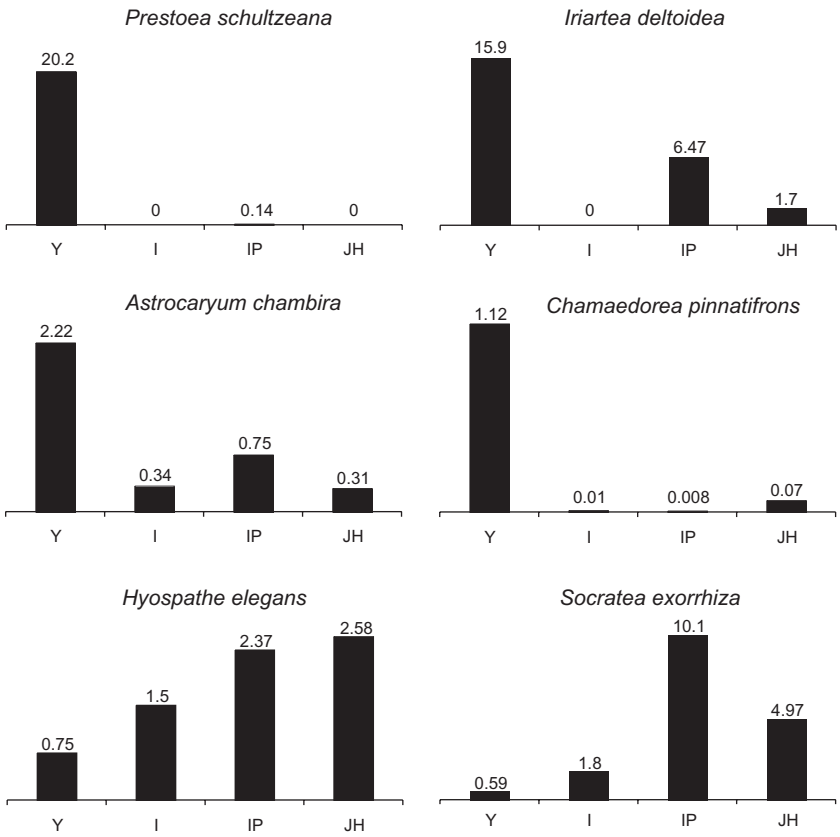

Figure 5. Variation in the abundance of palms in Intuto (I) and Jenaro Herrera (JH). Data for Iquitos–Pebas (IP) and Yasuní (Y) were taken from Vormisto *et al*. (2004a). Values on the solid bars represent the relative abundance (%) at each study site.

conditions, and that its edaphic preferences are opposite in Yasuní (association with well-drained soils) and Jenaro Herrera. In the latter locality, *O. bataua* grows mostly on hydromorphic or waterlogged soils, including seasonal swamps on upland valley floors, and on waterlogged gleyic podzols developed on white sands.

What can we expect from an intermediate area between Yasuní and Jenaro Herrera? Mantel tests and logistic regression showed an absence of correlation with the topography and drainage variables in Intuto. Juveniles and adult individuals of *O. bataua* grow both in seasonally flooded valleys and in well-drained uplands within this region.

These results could indicate that the ecology of *O. bataua* changes gradually across western Amazonia, although the sampled areas are small and relatively distant from each other. A greater geographical sampling is needed either to confirm this tendency or to unravel a more complex pattern. Another example of a possible ecological shift is illustrated by the distribution pattern of *Geonoma macrostachys* var. *acaulis*. In north-east Peru, this palm has been recorded as abundant in unflooded forest on hills (Vormisto *et al*., 2004b), whereas in Yasuní it grows within flooded forest or open vegetation on poorly drained soils. However, it was restricted to swamps in other localities of north-east Peru. It is again clear that a larger network of study sites is needed in order to understand better the complex effect of the spatial scale (local and regional) on ecological preferences of palms.

(iv) At a large scale, some palm distributions may be explained by ancient geological structures (ridge hypothesis) or more recent barriers (riverine barriers). The influence of ancient geological features (i.e. Iquitos Arch, Vaupés Arch, Serra do Moa Arch, Fitzcarrald Arch) on the geography of plants has been poorly explored. Recent evidence has revealed that these ancient features, which are not particularly prominent in the landscape, could play a significant role in the distribution of several taxa (Lougheed *et al*., 1999; Patton, da Silva & Malcolm, 2000).

Astrocaryum urostachys and *A. macrocalyx* can be used to illustrate this hypothesis. A close relationship between these two species has been found on the basis

of AFLP markers (Pintaud & Kahn, 2002). *Astrocaryum urostachys* has been considered as endemic to eastern Ecuador (Henderson, 1995; Borchsenius *et al*., 1998), and *A. macrocalyx* to north-east Peru (Kahn & Millán, 1992). We found that *A. urostachys* has a larger distribution range. We recorded a continuous distribution of *A. urostachys* on the floodplain of the Tigre River, from Intuto to its confluence with the Marañón River, in north-east Peru. Where *A. urostachys* meets *A. macrocalyx*, populations of both species form a very narrow sympatric zone, at the contact of the Marañon–Pastaza floodplain with the south-west basement of the Iquitos Arch. Within the hills of the Iquitos Arch, only *A. macrocalyx* occurs. Along the Napo River, *A. urostachys* grows abundantly from the lower Andes to the confluence of the Rio Napo with the Rio Curaray. A few kilometres downstream, the presence of hills indicates the north-east boundary of the Iquitos Arch, where again *A. macrocalyx* replaces *A. urostachys* (Fig. 5).

The contiguous distribution of these closely related species could be interpreted in two ways. The distribution could be a consequence of modern ecological constraints on these species, reflected mainly in the landscape topography (hilly vs. foodplains) or the evidence of a major floristic (and possibly faunistic) boundary corresponding to late Cenozoic palaeogeographical changes (arch or ridge hypothesis). Available data are still insufficient to favour one interpretation over the other. Gradual changes in species ecology, as described above, considerably complicate the interpretation of ecological constraints on species distribution in this region. This is also true for *Astrocaryum macrocalyx* and *Astrocaryum urostachys*. In distant, hilly areas of similar geomorphology (*A. urostachys* on the Andean Piedmont and *A. macrocalyx* on the Iquitos Arch), the two species appear to have exactly the same ecology, favouring hydromorphic valley bottoms (Vallejo, Vegas & Pintaud, 2004). In the known contact zones, by contrast, *Astrocaryum urostachys* is strictly associated with the floodplain, whereas *A. macrocalyx*, although favouring hills, is more ubiquitous and overlaps with *A. urostachys* on the floodplain.

More generally, macro-scale geological phenomena probably underlie the general edaphic conditions across the region studied. Vormisto *et al*. (2004a) found that soils in the Yasuní area were richer in nutrients than those of the Iquitos–Pebas area. If dispersal within the essentially continuous biota of western Amazonia is not a limiting factor (except in particular cases discussed below), edaphic specialization would largely explain the distribution pattern of palms observed in the region.

In order to evaluate the alternative hypothesis of a major vicariant event in the region of the Iquitos Arch and Miocene Pebas sea (Boeger & Kritsky, 2003), the distributional limits of more species of plants and animals must be documented to see if a common pattern emerges, independent of the ecology of individual species.

The role of fluvial systems in the Amazon Basin as barriers to gene flow between populations on opposite banks of the rivers (river hypothesis; Capparella, 1991) has been recently criticised (Lougheed *et al*., 1999; Patton *et al*., 2000), but it seems nevertheless to be relevant for the distribution of palms. The role of fluvial barriers is exemplified by the spatial distributions of *Astrocaryum macrocalyx* and *A. javarense* (Fig. 4). In the Iquitos region, the former species is located north of the Marañón River, whereas the latter is distributed south of the Marañon River. To the east, such a boundary occurs with *Oenocarpus distichus* (south of the Amazon River) and *O. bacaba* (north of the Amazon River), as pointed out by Henderson (1995). As these palms are dispersed by terrestrial mammals (Palacios, Rodriguez & Defler, 1997), a wide river represents an effective barrier to migration.

CONCLUSIONS

The first biogeographical hypothesis regarding palm distributions in Amazonia may be attributed to de Candolle (1857). He suggested that Amazonian palms were 'almost equally diffused throughout the tropics'. Huge advances in the systematics and ecology of Amazonian palms have been made since then. However, our understanding of the biogeography of palms has only slowly evolved, mainly because of a lack of precise species distribution data. In contrast with 19th century ideas, where the Amazon Basin was considered as a uniform ecosystem without major physical or floristic variations, modern scientific research reveals that Amazonia is a highly heterogeneous region, in which climate, soils, landscape, dispersal and geological history can only partially explain the distribution patterns of palms. Detailed ecogeographical data linked to well-resolved phylogenies are needed in order to understand the determinants of palm distribution at regional scales. Future studies should encompass new regions, as studies comparing few sites may reveal only a small part of the story.

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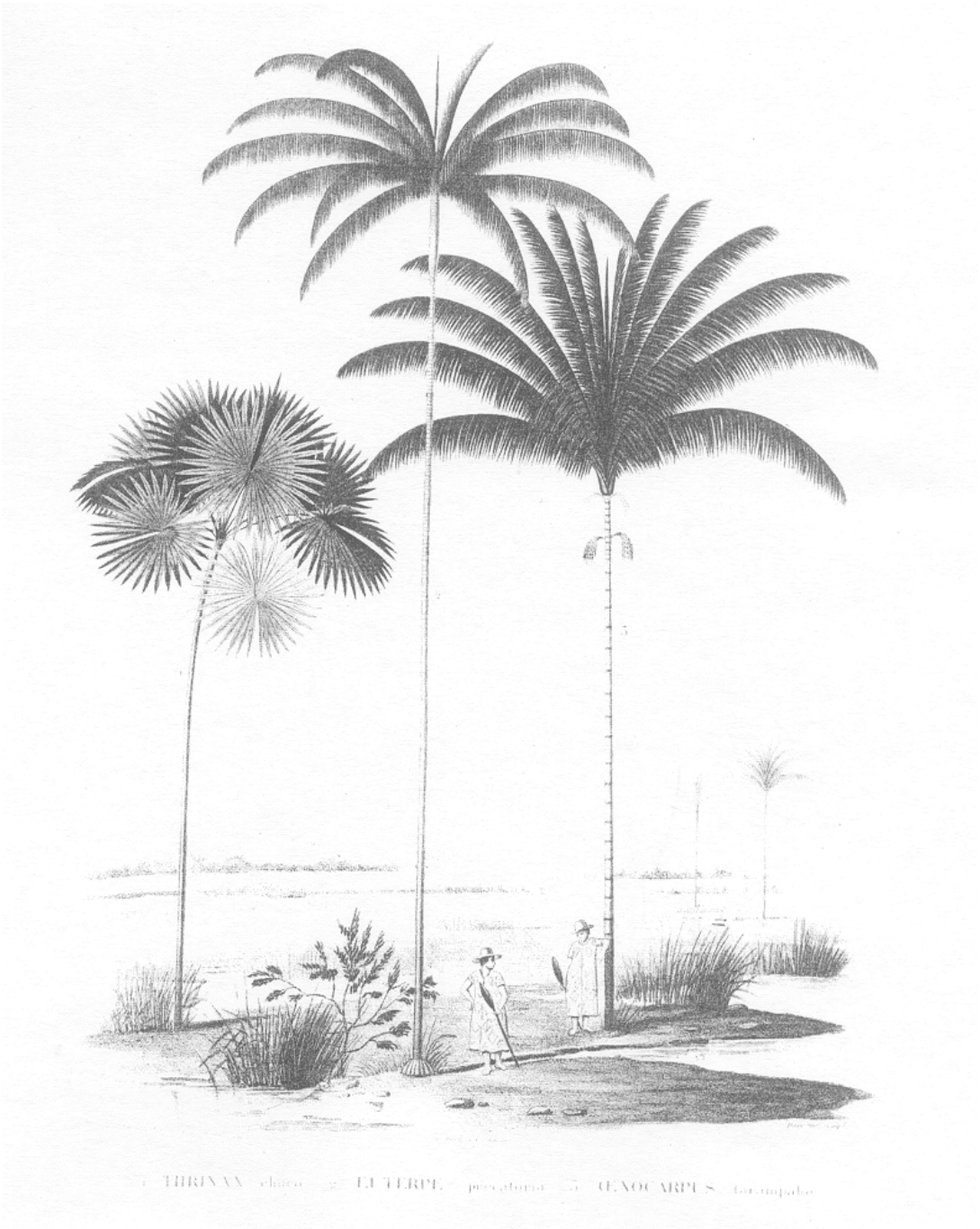
V

CONCLUSIONS ET PERSPECTIVES



V. CONCLUSIONS ET PERSPECTIVES

Notre étude, basée sur une approche multidisciplinaire (génétique des populations, chimiotaxinomie, morphométrie et écologie) avait pour but de valider ou rejeter les hypothèses taxinomiques sur (i) la définition des genres dans le complexe *Oenocarpus-Jessenia*, (ii) la définition des variétés d'*Oenocarpus bataua* Mart., et (iii) la différenciation génétique de populations écologiquement spécialisées. L'usage de plusieurs sources de données (marqueurs moléculaires, acides gras, caractères morphologiques et préférences écologiques) s'est avéré informatif à ces différents niveaux de structuration de la diversité biologique.

5.1. *Aspects taxinomiques*

5.1.1. Statut des genres *Oenocarpus* et *Jessenia*

Deux hypothèses sur la taxonomie des genres *Oenocarpus* et *Jessenia* ont été proposées: (i) Balick (1986) sépare *Jessenia* de *Oenocarpus*, et (ii) Henderson (1995) réduit *Jessenia* en synonymie de *Oenocarpus*. Ces hypothèses sont toutes deux basées sur l'interprétation des caractères morphologiques. Cependant, le nombre de caractères morphologiques distinctifs disponibles pour l'analyse demeure faible dans un genre petit et homogène comme *Oenocarpus*, ce qui limite la portée tant de l'interprétation classique (Balick 1986, Henderson 1995) que de l'analyse cladistique (Henderson 1999). L'usage d'une source de données additionnelle comme le sont les données moléculaires, apporte un éclairage nouveau sur cette problématique.

La phylogénie moléculaire de la tribu Euterpeae (Chapitre II) a montré l'absence de séparation entre *Oenocarpus* et *Jessenia*. Nos résultats montrent clairement que les individus de *Oenocarpus sensu stricto* et de *Jessenia* forment un groupe monophylétique soutenu par plusieurs synapomorphies, tandis que la différentiation entre espèces dans ce clade est très faible. Ce résultat est donc en accord avec l'étude phylogénétique de Henderson (1999), basée sur les caractères morphologiques et anatomiques.

Bien que notre analyse phylogénétique soit basée sur plus de 3,6 kb de séquences chloroplastiques, sa résolution est limitée par la très faible variabilité nucléotidique dans le

groupe étudié. Un faible taux de mutation a été suggéré pour le chloroplaste des palmiers en général (Wilson *et al.* 1990). Cette évolution semble encore plus lente que la moyenne dans la tribu Euterpeae. Les séquences des espaceurs chloroplastiques analysées ont apporté à peine 1.5% de caractères informatifs pour construire la phylogénie. Cette valeur est faible en comparaison des valeurs > 5% indiquées par Asmussen et Chase (2001) et Hahn (2002) pour des séquences chloroplastiques non-codantes. Sur les 3630 positions alignées, à peine 49 substitutions potentiellement informatives pour la phylogénie, quatre inversions et trois indels ont été détectées. Malgré le faible pourcentage de caractères informatifs, les séquences chloroplastiques utilisées nous ont permis de mettre en évidence quelques relations, en particulier de clarifier le statut de *Jessenia*, de mettre en évidence le genre frère de *Oenocarpus* (*Euterpe*) et de confirmer la monophylie de la tribu Euterpeae. Les séquences chloroplastiques ont cependant montré leur limite comme source d'information phylogénétique au niveau de genres proches et entre espèces chez les palmiers et dans d'autres familles végétales (Asmussen 1999, Baker *et al*. 1999). À l'avenir, il conviendra sans doute de séquencer des régions nucléaires pour aller plus loin dans la connaissance de la phylogénie des Euterpeae, et particulièrement pour tester la monophylie d'*Oenocarpus bataua*.

5.1.2. Taxinomie infraspécifique de *Oenocarpus bataua* Mart.

Dans l'analyse de phylogénie moléculaire basée sur les marqueurs chloroplastiques, les échantillons représentant les variétés *bataua* et *oligocarpa* ne se regroupent pas ensemble dans le clade de *Oenocarpus*, ce qui fut un résultat inattendu, mais finalement en accord avec la différentiation montrée par l'analyse AFLP (Chapitre II). Le polymorphisme d'inversion rencontré dans la région *psbC-trnS* et génotypé chez 10 individus de chaque variété corrobore l'information des substitutions en distinguant clairement chacune des variétés. De même que les séquences chloroplastiques, les marqueurs AFLP ont donc détecté une forte différenciation génétique associée à la taxinomie infraspécifique et à la répartition géographique des populations. Cette différenciation se traduit par les valeurs F_{ST} (Phi values) très élevées (> 0.5) entre variétés, et par le fait que l'AMOVA indique que la variance interpopulationnelle est supérieure à la variance intrapopulationnelle.

L'isolement par la distance pourrait être une explication simple à la différentiation entre les populations de *Oenocarpus bataua* étudiées, représentant les deux variétés. Les

populations situées de chaque côté des Andes sont plus proches géographiquement et génétiquement entre elles qu'elles ne le sont de la population de Guyane. Nous aurions là une explication qui ne nécessite pas de prendre en compte la taxinomie infraspécifique. Cependant, il est peu probable que ce schéma resterait valide si l'on pouvait échantillonner dans l'ensemble du bassin amazonien. En effet, il existe des populations de *O. bataua* var. *bataua* en Amazonie orientale, jusque sur l'île de Marajo dans l'embouchure de l'Amazone (Henderson *et al.* 1991), géographiquement proches des populations de *O. bataua var. oligocarpa* des Guyane. En certains endroits de la Guyane Française, les deux variétés semblent même entrer en contact (Granville 2002).

Les données biochimiques (acides gras du mésocarpe) montrent également des variations qualitatives et quantitatives importantes entre *O. bataua var. bataua* et *O. bataua var. oligocarpa* (Chapitre III-*a*). Par exemple, une plus grande diversité d'acides gras minoritaires (traces) a été détectée dans les échantillons de *var. bataua*, et des différences significatives dans la teneur en acides gras majoritaires ont été détectées entre les deux variétés. L'origine de la variation biochimique est mal connue et peut être liée à (i) des caractéristiques génétiques intrinsèques à chacune des variétés et (ii) à une réponse à des variations de l'environnement. On notera en particulier que les substrats géologiques de l'Amazonie occidentale et du bouclier guyanais sont totalement différents.

Enfin, nous disposons d'un jeu de données morphologiques acquis directement sur le terrain, et différent des caractères d'herbiers utilisés précédemment pour distinguer les deux variétés (Chapitre III-*b*). Six des huit caractères reproductifs et deux des quatre caractères végétatifs que nous avons étudiés montrent des différences significatives entre variétés. En particulier, le nombre de rachéoles et de pennes, ainsi que la longueur du rachis floral et l'abondance de fibres à la base des feuilles, ont été les caractères les plus discriminants entre var. *bataua* et var. *oligocarpa*. Malgré des limitations techniques pour obtenir du matériel fertile des populations naturelles de *O. bataua*, les caractères mesurés s'avèrent tout à fait informatifs mais la différentiation entre les deux variétés correspond plus à des variations quantitatives que qualitatives.

Nos résultats ont donc mis en évidence une différenciation génétique, biochimique et morphologique entre les deux variétés. Il est plus difficile de comparer ces deux variétés au niveau écologique, vu qu'elles sont associées à des formations géologiques bien distinctes

(la vallée de l'Amazone et le bouclier Guyanais). On peut noter cependant que les populations de var. *bataua* tendent à présenter des spécialisations au niveau du micro-habitat, tandis que les populations de var. *oligocarpa* sont plutôt généralistes (Kahn et Granville 1992). Les caractéristiques morphologiques, écologiques, géographiques et les différences génétiques contribuent fortement à la définition de l'espèce (Prat *et al*. 2006), donc nous pouvons suggérer que var. *bataua* et var. *oligocarpa* pourraient représenter plutôt des espèces différentes que des catégories infraspecifiques. Cette hypothèse a été déjà proposée par Grisebach et Wendland (1864 in Balick 1986) et Wessels Boer (1965).

Au total, il semble que nous ayons à faire à un cas de vicariance biogéographique entre les populations de l'Amazonie occidentale (*var. bataua*) et celles des Guyanes (*var. oligocarpa*), comme cela a déjà été documenté pour de nombreux groupes de plantes et d'animaux (Terborgh et Andresen 1998, Colinvaux et De Oliveira 2001, terSteege *et al.* 2006).

5.2. *Dynamique de la diversité génétique de Oenocarpus bataua var. bataua dans l'Amazonie occidentale*

Les populations naturelles des espèces forestières sont caractérisées en général par une forte variabilité génétique souvent peu structurée (Prat *et al*. 2006). C'est le cas de *Oenocarpus bataua* (Chapitre IV-*a*), chez qui apparaît une grande diversité allélique à tous les locus microsatellite analysées (jusqu'à 19 allèles pour le locus Ob19). De plus, la quasi-totalité de la diversité génétique (90%) se situe au niveau intrapopulationnel.

La grande diversité et faible structuration génétique des populations forestières de *Oenocarpus bataua* sont liées à des facteurs écologiques et historiques.

(i) *Oenocarpus bataua* est une espèce préférentiellement allogame. Le système reproductif a une influence directe sur la diversité et la structure génétique (Loveless et Hamrick 1984). L'allogamie favorise l'homogénéisation interpopulationnelle et tend à faire augmenter la diversité totale (Hamrick *et al.* 1992, Loveless 1992, Hamrick et Godt 1996).

(ii) La zoochorie, et particulièrement l'ornithochorie permet la migration des graines de *Oenocarpus bataua* à relativement longue distance (Snow et Snow 1978, Sist et Puig 1987,

Balick 1988). Snow et Snow (1978) ont indiqué que l'oiseau *Steatornis caripensis*, une espèce largement répandu dans la forêt amazonienne, consomme et transporte les semences de *O. bataua* jusqu'à 30 miles (48 kilomètres) de l'arbre source. Si cette tendance à la dispersion des graines est continuellement répétée tout au long de l'aire de distribution de *O. bataua*, la dissémination des graines par *S. caripensis* favorise le flux génétique entre populations et en conséquence l'homogénéisation de la diversité génétique des populations de *O. bataua*. D'autres espèces d'oiseaux comme les toucans, les perroquets et les marailles (*Penelope purpurascens*) sont aussi des disséminateurs des graines de *O. bataua* (Sist et Puig 1987). Chez *O. bataua*, l'influence des oiseaux dans le flux de gènes n'a pas été spécifiquement étudiée; cependant l'ornithochorie pourrait être une force majeure dans la structuration génétique de l'espèce.

(iii) Les données archéologiques suggèrent que l'usage des fruits de palmiers par l'homme en l'Amazonie est important depuis au moins 9000 BP (Morcote Rios et Bernal 2001). Durant cette période, l'homme nomade chasseur-cueilleur pourrait avoir influencé la structure de la diversité de *O. bataua*. Encore aujourd'hui, les Amérindiens de l'Amazonie transportent les fruits de *O. bataua* pour leur consommation pendant leurs déplacements dans la forêt.

(iv) *Oenocarpus bataua* présente une vaste distribution géographique. L'effet de la taille de l'aire de répartition d'une espèce sur la diversité et la structuration génétique a été largement étudié (Hamrick *et al.* 1992, Hamrick et Godt 1996). La diversité et la structure génétique des populations ou espèces localement endémiques ayant des effectifs réduits sont influencées par les effets de la dérive génétique. Cette force évolutive favorisera la perte d'allèles et en conséquence la réduction de la diversité génétique. Par contre, dans une grande aire de répartition, la diversité génétique peut se maintenir plus facilement (Hamrick et Godt 1996, Hamrick *et al.* 1992). *Oenocarpus bataua* est largement et régulièrement distribué dans l'Amazonie et les Guyanes, ce qui pourrait favoriser un intense flux des gènes par le biais des pollinisateurs et des disséminateurs.

(v) La diversité importante des espèces forestières de l'Amazonie est la conséquence des bouleversements ou évènements géologiques ayant affecté l'histoire de ces populations et de facteurs ayant favorisé la conservation de la diversité produite au cours des temps géologiques. La région nord de l'Amazonie occidentale, ou région du Napo, a été proposée

comme un refuge pour les populations végétales pendant les phases froides du Pléistocène et post-Pléistocène (Haffer 1969, 1982, 1997). La dynamique des populations naturelles dans cette région pendant le Pléistocène demeure mal connue; cependant, de multiples évènements d'expansion et rétraction des populations naturelles pourraient avoir laissé leur marque sur la structure génétique des populations de *Oenocarpus bataua*. En outre, l'influence d'une mer intérieure sur une grande partie de l'Amazonie occidentale au Miocène (Mer de Pebas, Frailey *et al.* 1988), ou l'impact des soulèvements tectoniques comme l'arche d'Iquitos (Roddaz *et al.* 2005), sur les populations forestières, sont peu étudiés. Nos résultats (Chapitre IV-*b*) montrent qu'au moins pour les populations de palmiers, la diversité d'espèces et d'écotypes pourrait être associée à l'histoire géologique de la région (Montufar et Pintaud 2006).

5.3. *Variabilité écologique de Oenocarpus bataua var bataua*

Oenocarpus bataua Mart. est l'un des palmiers les plus abondants et représentatifs de l'Amazonie et de la région des Guyanes (Sist et Puig 1987, Kahn 1991, ter Steege *et al.* 2006). La présence de *O. bataua* est généralement associée à des sols hydromorphes (marais) dans l'Amazonie (Kahn et Granville 1992, Ribero *et al*. 1999). Cette espèce a été utilisée comme un indicateur biologique de forêts sur sols mal drainés (Encarnación 1985, Kahn et Granville 1992).

Le chapitre IV-*b* montre que les patrons de distribution à l'échelle du micro-habitat de *O. bataua* sont en fait assez variables. On peut dire que la distribution de *O. bataua* présente un *ecocline* associé aux conditions édaphiques. Les extrêmes géographiques correspondent à des populations spécialisées pour des conditions édaphiques différents (sols bien drainés *vs*. hydromorphes), tandis que les populations géographiquement intermédiaires sont généralistes par rapport aux conditions édaphiques.

Les bases génétiques de cette différenciation écologique ont fait l'objet d'une étude préliminaire dans le chapitre IV-*a*. Huit marqueurs microsatellites ont été utilisés pour explorer la structure génétique de trois populations écologiquement différenciées de *Oenocarpus bataua* dans l'Amazonie occidentale. Nos résultats n'ont révélé aucune trace de structuration génétique associée à la différenciation écologique. Il est possible que la différenciation écologique soit un évènement historiquement récent qui ne montre pas de

trace sur les marqueurs neutres comme les microsatellites, ou que les marqueurs microsatellites sont de faibles indicateurs de la spécialisation éco-physiologique, ou que des facteurs externes comme la compétition interspécifique entre en jeu.

5.4. *Perspectives de conservation et de valorisation*

5.4.1. Valorisation de l'huile de *Oenocarpus bataua* Mart.

Les restrictions à la commercialisation de l'huile d'olive pendant la première et seconde guerre mondiale (1914-1945) ont poussé la recherche de nouvelles sources d'huiles comestibles. À partir de la première guerre mondiale, plusieurs programmes de recherche ont été mis en place pour explorer la forêt tropicale et répertorier les espèces oléagineuses (Pesce 1985, Cavalcante 1974, Hodge 1975, Cavalcante et Johnson 1977, Balick 1988, Kahn 1988).

Parmi les espèces oléagineuses de l'Amazonie, les palmiers présentent un intérêt économique et nutritionnel majeur. En particulier, une vaste littérature scientifique à mis en évidence le potentiel économique de *O. bataua* comme une source d'huile comestible de haute qualité pour la consommation humaine (Balick 1986, 1988). Toutefois, aucun projet commercial n'a été développé dans ce sens à ce jour. Il n'existe que des programmes pilotes pour le développement d'une industrie locale de l'huile de *O. bataua* et *O. bacaba* à partir des populations sauvages, en Colombie (Balick 1986), et au Brésil. Notre étude apporte des informations biochimiques nouvelles concernant le potentiel nutritionnel et économique de cette ressource naturelle négligée de l'Amazonie. Aujourd'hui, la recherche scientifique sur les espèces oléagineuses présente un intérêt majeur de par ses implications dans les domaines nutritionnel et médical.

Les études sur la composition en acides gras du mésocarpe des fruits de *Oenocarpus bataua* réalisées précédemment ont été conduites sur un nombre limité d'échantillons dont l'identité n'est pas garantie (Balick et Gershoff 1981, Lubrano et Robin 1997, Alemán *et al*. 2002, Esriche *et al*. 1999). Le chapitre III-*c* fait état d'une nouvelle description de la composition en acides gras, basée cette fois sur un échantillonnage important de plusieurs populations de Guyane et de l'Amazonie occidentale. Notre étude a mis en évidence la haute teneur en acides gras insaturés du mésocarpe, en particulier en acide oléique. En

comparaison des huiles végétales largement consommées dans la zone tropicale comme l'huile de palme (*Elaeis guineensis*) ou de coprah (*Cocos nucifera*), l'huile de *O. bataua* est un produit beaucoup plus indiqué pour la santé humaine et en particulier pour la prévention des maladies cardio-vasculaires, en raison de sa faible teneur en acides gras saturés et de la présence de plusieurs acides gras polyinsaturés de type omega 3 et 6.

La variabilité biochimique des fruits de *Oenocarpus bataua* est critère de première importance à prendre en compte pour de futurs programmes de domestication de l'espèce. La variabilité biochimique n'a pas été décrite dans la littérature. Le chapitre III-*c* fait état de la remarquable variabilité biochimique (acides gras et teneur en lipides) entre les variétés *oligocarpa* et *bataua*. Par exemple, un important caractère agronomique lié à la productivité comme la teneur en lipides du mésocarpe, varie de 33.6 % à 63% entre les populations du Pérou et la Guyane française. Ces résultats montrent une large gamme de variabilité biochimique qui pourrait être utilisée pour l'amélioration de cette ressource.

5.4.2. Conservation de *Oenocarpus bataua*

Bien qu'aujourd'hui *Oenocarpus bataua* soit loin d'être considéré comme une espèce menacée, en raison de sa vaste aire de distribution et de son abondance dans les écosystèmes forestiers, on peut prévoir qu'à moyen terme, le déboisement de la forêt et le système actuel d'exploitation des fruits (extractivisme destructeur) conduira à l'érosion de la diversité génétique des populations sauvages, matière première pour l'amélioration génétique de l'espèce.

L'industrialisation de *Oenocarpus bataua* devra s'appuyer sur le développement (i) de plantations et (ii) sur un programme international pour l'amélioration génétique de l'espèce. La récolte des fruits à partir des populations naturelles n'est pas économiquement rentable à cause des fluctuations dans la production des fruits et la difficulté d'accès aux arbres. Dans ce contexte, l'installation de cultures intensives ou intégrées dans systèmes agroforestiers est une priorité qui permettrait à la fois de réduire la pression sur les populations naturelles et d'augmenter la productivité. La limitation majeure en culture est la lenteur de croissance de ces palmiers (Kahn & Granville 1992).

La domestication de *Oenocarpus bataua* constitue un défi pour les biologistes et cultivateurs de la région amazonienne. À l'exception de *Bactris gasipaes*, aucun palmier néotropical n'a été soumis à un processus de domestication par les peuples amérindiens. Il est probable que l'abondance de la ressource dans la forêt n'ait pas favorisé l'intérêt pour la domestication de *O. bataua*. De plus, les caractéristiques du fruit sont naturellement satisfaisantes pour les usages qui en sont faits. Les programmes de domestication de *O. bataua* devraient être dirigés vers l'exploration de la variabilité naturelle de l'espèce ainsi que sur l'obtention d'hybrides. L'existence d'hybrides inter-spécifiques chez *Oenocarpus* est mentionnée dans la littérature (Balick 1986). Par exemple, les hybrides *O. bataua* × *O. bacaba* donnent des fruits stériles plus charnus que ceux de *O. bataua*. Ces hybrides constituent une avancé en matière de productivité. De plus, les fruits de *O. bataua* provenant de l'Amazonie occidentale sont plus riches en acides gras insaturées que les populations des Guyanes. Les croisements inter-populations pourraient également améliorer la composition biochimique des fruits.

5.3. *Conclusion générale*

Durant plus d'un siècle, le statut du genre *Jessenia* a été conflictuel. De nombreuses publications ont utilisé et utilisent encore différents noms scientifiques pour faire référence à un même taxon. Nos résultats démontrent définitivement l'inclusion du taxon *Jessenia bataua* dans le genre *Oenocarpu*s.

Ce travail inclut la première phylogénie moléculaire de la tribu Euterpeae. Outre le positionnement de *Jessenia*, nous avons pu montrer que *Oenocarpus* est le genre frère d'*Euterpe*, ce qui n'avait jamais été suggéré par l'analyse morphologique, *Euterpe* ayant été considéré comme proche de *Prestoea*. Cependant, il reste encore de nombreuses questions à résoudre au sein des Euterpeae, en particulier en ce qui concerne les relations intergénériques, et également les relations interspécifiques dans le genre *Oenocarpus*. Dans cette optique, l'ajout des espèces manquantes dans la phylogénie, en particulier *O. makeru, O. simplex* et *O. circumtextus*,et le séquençage de nouvelles régions génomiques sont une nécessité, et cela pourrait permettre de déterminer si *Jessenia* mérite un statut de sous-genre. De futures études moléculaires pourraient également s'interesser à l'hybridation dans le genre *Oenocarpus*, certains hybrides (*bataua* × *mapora*, *bataua* × *bacaba*) ayant un potentiel agronomique.

Notre étude fait état d'une importante différenciation moléculaire, biochimique et morphologique entre les variétés *bataua* et *oligocarpa*. Ce résultat va dans le sens du point de vue taxinomique de Grisebach et Wendland (1864) qui considère ces deux variétés comme des espèces différentes. La question ne peut néanmoins être tranchée car nous manquons de résolution phylogénétique dans le genre *Oenocarpus*.

Oenocarpus bataua est préférentiellement un palmier des régions tropicales de basse altitude, cependant certaines populations peuvent atteindre 1350 mètres d'altitude sur les pentes andines. Ces populations sont plutôt rares et n'ont pas été étudiées à détail au niveau morphologique ou génétique. Burret (1928) a décrit l'espèce *Jessenia weberbaueri* provenant d'une population à 900 mètres d'altitude dans la région de Moyobamba (Pérou). Cette espèce a été mise en synonymie de *Oenocarpus bataua* en dépit de variations morphologiques au niveau de l'inflorescence. Les populations andines sont également une ressource génétique de grande valeur agronomique puisqu'elles pourraient êtres utilisées pour améliorer l'adaptation aux régions subtropicales.

En Amazonie, *Oenocarpus bataua* comme d'autres espèces sauvages de la forêt tropicale montre une forte diversité génétique mais une faible différentiation interpopulationnelle à l'échelle régionale. La différenciation écologique chez les populations de *O. bataua* dans l'Amazonie occidentale n'est pas liée à une structuration génétique observée à l'aide des marqueurs neutres. Le déterminisme de la divergence écologique demeure à explorer.

VI

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VII

ANNEXES

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PRIMER NOTE

Isolation of 23 polymorphic microsatellite loci in the Neotropical palm *Oenocarpus bataua* Martius (Arecaceae)

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Abstract

We report the isolation of 23 microsatellite loci obtained from a $(GA)_n$-enriched genomic library of *Oenocarpus bataua* var. *bataua*. The average number of alleles per locus, the mean observed and expected heterozygosities for these microsatellite loci revealed a high level of variability. The transferability of the developed markers to other *Oenocarpus* species and other genera within the Euterpeae tribe was high, except for the *Hyospathe* genus.

Keywords: Amazon, Arecaceae, Euterpeae, microsatellites, *Oenocarpus bataua* var. *bataua*

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The tribe Euterpeae (Arecaceae) is composed of five genera (Dransfield *et al*. 2005) and 31 species distributed in the Neotropics (Govaerts & Dransfield 2005). This palm group includes several economically important species, some of which are sources of vegetable oil (*Oenocarpus* spp.), palm hearts (*Euterpe* spp., *Prestoea acuminata*) and edible fruits (*Oenocarpus* and *Euterpe* species). *Oenocarpus bataua* is a monoecious solitary palm that grows up to 25–30 m tall, and it is common throughout the Amazon basin. Its fruits are rich in oleaginous compounds, and have long been used as a source of edible oil by indigenous communities (Balick 1986). Despite its importance as a potential new crop in the region, its genetic variability has not been studied. Here we report on (i) the isolation of 23 microsatellite markers from *Oenocarpus bataua* var. *bataua* that will be used for a preliminary genetic analysis and (ii) a cross-species transferability experiment within the Euterpeae tribe.

Total genomic DNA was extracted using the QIAquick PCR purification kit (QIAGEN). DNA was digested with *Rsa*I restriction enzyme and latterly ligated to adaptors. The DNA fragments containing microsatellite $(GA)_n$ were hybridized with a biotin-labelled oligoprobe followed by the capture of selected sequences with streptavidin-coated magnetic beads (Billotte *et al*. 1999). Selected fragments were cloned into a pGEM-T Easy Vector (Promega) and transformed in competent *Escherichia coli* XL1-Blue cells following the manufacturer's instructions. One hundred and seventy-nine transformant colonies (clones) were recovered and diluted in 30 μL of water. We attempted to identify clones with a $(GA)_n$ motif by amplifying the plasmid's insert using standard SP6 and T7 promoter primers and a forward primer $(GA)_{15}$. Clones were amplified using polymerase chain reaction (PCR) in 20 μL containing 5 μL of the former dilution (clone), 370 μM dNTP mix, 1 U of *Taq* DNA polymerase, 1× of PCR reaction buffer (Colorless Go-*Taq*, Promega), 2 μM $MgCl_2$, 0.15 μM of each promoter primers (SP6, T7) and 0.15 μM of the forward primer $(GA)_{15}$. The thermal cycling profile was 2 min of initial denaturation at 96 °C followed by 35 cycles at 95 °C for 30 s, 55 °C annealing temperature for 30 s, and 72 °C elongation step for 45 s. A final 10-min extension step at 72 °C was added. The amplified products were run on 1% agarose electrophoresis gels. The band patterns on agarose gel showed: (i) a major band amplified by SP6 and T7 primers (total insert) with an average size of 846 bp; and (ii) one or many minor bands (< 846 bp) amplified by the forward primer $(GA)_{15}$ and one of the two promoter primers. The presence of at least one minor band was interpreted as evidence of a $(GA)_n$ motif. Each sequenced clone confirmed this postulate.

The size of major and minor bands enabled us to estimate the position of the $(GA)_n$ motif in the insert. Minor bands with a size close to the total insert length or very small (< 100 bp) suggested the presence of a $(GA)_n$ motif close to the 5′ and 3′ end, respectively, and therefore not suitable to design primers on the flanking regions of the sequences. On the other hand, minor bands ranging from 600 to 300 bp suggested the presence of a $(GA)_n$ motif in a central position of the insert, which was suitable for primer

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Table 1 Information on the 23 microsatellite loci isolated from *Oenocarpus bataua* var. *bataua*. The final annealing temperature for all loci was 55 °C. The number of alleles (N_A) was calculated on basis of 15 and 35 *O. bataua* samples; observed (H_O) and expected (H_E) heterozygosities and fixation index (F_{IS}; significance level *$P < 0.05$) were calculated on basis of 35 *O. bataua* samples. A M13 tail (CACGACGTTGTAAAACGAC) was attached at the 5′ position of the forward primer for all loci

				Oenocarpus bataua					
				15 samples	35 samples				GenBank Accession no.
Locus	Repeat array	Primer sequence (5′–3′)	Size (bp)	N_A	N_A	H_E	H_O	F_{IS}	
Ob01	$(GA)_{17}$	F: TTTGAGTTCCCCAAATCTAATACA R: GGATGAGAGGCAAGGCATAA	180–220	8	14	0.76	0.85	−0.11	DQ455752
Ob02	$(GA)_{20}(CA)_{10}$	F: CTGAACCTTATCCCAACTGA R: CACATAACTTTTCAGGCACA	128–153	7	—	—	—	—	DQ455753
Ob03	$(GA)_{14}$	F: ATTGTTTCCAGTCATCATCC R: TTGCAAGACAATTTCGTAGA	110–133	10	10	0.79	0.79	0.090	DQ455754
Ob04	$(GA)_{17}$	F: CCATATACGGGCAAATTAAG R: CATGTGAACACGCTAGGAG	145–190	11	—	—	—	—	DQ455755
Ob05	$(GA)_{15}$	F: GGATTCTATGAGAACATACCC R: GAGGTAGGCTAGGCCTAAAG	160–190	4	—	—	—	—	DQ455756
Ob06	$(GA)_{17}$	F: GGATTGCATGTGTTCATTTA R: TTACGCAATGTTTTATTTGG	200–235	11	10	0.86	0.87	−0.005	DQ455757
Ob07	$(GA)_{13}$	F: ATGGCAGTGCTTTGATATTC R: TTTAATGGAGGGTTTGATTG	180–213	10	13	0.88	0.73	0.158*	DQ455758
Ob08	$(GA)_{15}GG(T)_{24}$	F: GAGGAGGAATTCTTTCCATT R: AGCCATTAAAATTCATGCAC	206–230	7	—	—	—	—	DQ455759
Ob09	$(GA)_{14}$	F: AGTCCGTAAAACAGGATCAA R: GGATGGATCCTTCTTCTCAT	178–193	6	—	—	—	—	DQ455760
Ob10	$(GA)_{19}$	F: GCCTTCTTCCTCCCTATCT R: TCCAAGATACCGAATCTCAC	153–193	10	—	—	—	—	DQ455761
Ob11	$(GA)_{14}$	F: ATGAGGGATGTCAATGGAT R: AAAATTCTCCTCTCGCTCTT	145–185	14	—	—	—	—	DQ455762
Ob12	$(GA)_{10}AC(GA)_9$	F: GTTCAGAGAAGGATCTGGTG R: GGGAAAGAAAAAGAGAGGAG	178–225	9	—	—	—	—	DQ455763
Ob13	$(GA)_{18}$	F: CCTATCCCCCTGAACTCTAT R: GAGTTGGTGAAGGACTCAGA	170–208	13	—	—	—	—	DQ455764
Ob14	$(GA)_{13}$	F: TGGCATTCTTGACTTTGCAT R: ACCATGCCAACTGTGACCTT	160–178	3	7	0.59	0.67	−0.073*	DQ455765
Ob15	$(GA)_{10}$	F: CTCTTCTTCCATCCCTTTTT R: TTTTCAGAGGGTATGAGGTG	215–243	9	10	0.72	0.70	0.018	DQ455766
Ob16	$(GA)_{16}$	F: AGGTCTAATGATGGAAGCTG R: ACACAGAGACAAACATGTGC	135–193	17	—	—	—	—	DQ455767
Ob17	$(GA)_{16}$	F: TAGCTTTAGAGCGAGGGACT R: TGAGCCATAGAACTGACCTT	140–160	6	10	0.83	0.72	0.133	DQ455768
Ob18	$(GA)_{18}$	F: GCTCCAGCTTCCAAGATAC R: ACAAGTGACTGTTCCTCACC	128–160	7	13	0.79	0.84	−0.074	DQ455769
Ob19	$(GA)_{18}$	F: CCGAATCTCACCTAAACAAG R: CACCACCTAACACTTTCTTTG	193–233	11	18	0.84	0.88	−0.042	DQ455770
Ob20	$(GA)_{14}$	F: CATGAATTAGCTGCGGTGTG R: AAGCACACCCGAGAAAGAAA	170–193	6	7	0.73	0.67	0.088	DQ455771
Ob21	$(GA)_9$-$(GA)_{16}$	F: AGAGTGCTAGGGGTGCTCAT R: TGAAATGGTTGATGAATTGAATG	213–245	14	13	0.88	0.81	0.079	DQ455772
Ob22	$(GA)_5$-$(GATA)_9$ $(GA)_{17}$	F: TTTGGATTGTCAAAACCACTG R: ATCTCTTGCTTGCGGTCAAT	190–233	13	—	—	—	—	DQ455773
Ob23	$(GA)_{20}$	F: GCCAAGTTGGAAGAAAGAAT R: CTCCTCTGATCTCTGTTTGG	150–178	10	—	—	—	—	DQ455774

design. A total of 50 clones, showing a $(GA)_n$ motif in a central position of the insert, were sequenced in the two directions using an ABI PRISM 3130xL Genetic Analyser (Applied Biosystems).

Sequence edition, alignment and redundancy assessments were performed with LASERGENE 5.1 software (DNAStar Inc.). Thirty-five of 50 sequences allowed us to design primers. Amplification was successful for 23 of them. Primers were

designed using the Web-based PRIMER3 software program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Forward primers were synthesized with a 5′-M13 (CACGACGTTGTAAAACGAC) tail for use in the universal dye-labelling method (Boutin-Ganache *et al*. 2001).

Twenty-three microsatellite loci were first screened on a set of 15 DNA samples of *O. bataua* (four samples from French Guiana and 11 from Peru). PCRs were performed in a 15-μL total volume, with 125 μM dNTP mix, 1 U of *Taq* DNA polymerase, 1× PCR reaction buffer (Colorless Go-*Taq*, Promega), 2.5 mM $MgCl_2$, 0.04 μM forward 5′M13 primer, 0.1 μM reverse primer, and 0.1 μM M13 fluorescent-labelled (IRD-700) primer. The thermal cycling profile for all 23 microsatellite loci was described above. PCR products were diluted 1:20 with the stop/loading formamide buffer and electrophoresed on the LI-COR 4200 DNA analysis system.

A second set of 35 DNA samples of *O. bataua* from the locality of Intuto in NE Peru was genotyped with 11 loci. PCRs and the thermal cycling follow the previous protocol. Amplified products were detected with an ABI PRISM 3130xL Genetic Analyser (Applied Biosystems). Allelic patterns were analysed using GENEMAPPER 3.7 (Applied Biosystems). Heterogozity values were computed with POWERMARKER 3.25 (Liu & Muse 2005); Hardy–Weinberg equilibrium (HWE), fixation index (F_{IS}) and linkage disequilibrium were calculated with GENEPOP 3.1 (Raymond & Rousset 1995).

For the first data set, 23 loci gave a positive signal in the LI-COR sequencer. These microsatellite markers were polymorphic with three to 17 alleles and a mean of 9.4 alleles per locus (Table 1). For the second data set, the allele number per locus varied from seven to 14 with a mean of 11.4. No evidence of linkage equilibrium was detected, and all loci were in HWE, except for Ob07 and Ob14 loci. The observed heterozygosity ranged from 0.67 to 0.88 with a mean of 0.74, and the expected heterozygosity ranged from 0.59 to 0.88, with a mean of 0.79 (Table 1).

The transferability of these 23 novel microsatellite markers was tested across six *Oenocarpus* species and four species representing each of the other Euterpeae genera. The transferability of microsatellite loci was high among *Oenocarpus* species. The 23 microsatellite markers of *O. bataua* var. *bataua* were successfully transferred to *O. bataua* var. *oligocarpa* and at least 90% of them were transferred to *Oenocarpus mapora*, *Oenocarpus minor*, *Oenocarpus distichus*, 87% to *Oenocarpus bacaba* and 78% to *Oenocarpus balickii*. The transferability to other genera was also high (83% for *Euterpe* and *Neonicholsonia*, and 78% for *Prestoea*), with the exception of *Hyospathe* for which only 48% of microsatellite markers gave a positive signal (Table 2).

Table 2 Interspecific amplification of 23 microsatellite markers isolated from *Oenocarpus bataua* var. *bataua* within the Euterpeae tribe. Successful amplification (+) or weak/failed amplification (–) is indicated for 10 taxa each represented by only one DNA sample (*O. Oenocarpus* species). Values in last column represent the size range (bp) of the bands detected

Locus	*O. bataua oligocarpa*	*O. bacaba*	*O. balickii*	*O. mapora*	*O. minor*	*O. distichus*	*Euterpe oleracea*	*Hyospathe elegans*	*Prestoea acuminata*	*Neonicholsonia watsonii*	Size range (bp)
Ob01	+	+	+	+	+	+	+	–	+	+	180–230
Ob02	+	+	+	+	+	+	+	–	+	+	130–230
Ob03	+	+	+	+	+	+	+	–	+	+	120–170
Ob04	+	–	–	+	–	–	+	–	–	+	170–240
Ob05	+	–	–	–	–	+	+	–	–	–	180–230
Ob06	+	+	+	+	+	+	+	–	–	+	180–220
Ob07	+	+	+	+	+	+	+	+	+	+	180–230
Ob08	+	+	–	+	+	+	+	–	+	+	210–240
Ob09	+	+	+	+	+	+	+	+	+	+	170–213
Ob10	+	+	+	+	+	+	–	–	+	–	180–234
Ob11	+	+	+	+	+	+	+	+	+	+	150–230
Ob12	+	–	+	+	+	+	+	–	+	+	170–220
Ob13	+	+	+	+	+	+	+	+	+	+	120–190
Ob14	+	+	+	+	+	+	+	+	+	+	180–200
Ob15	+	+	+	+	+	+	+	+	+	+	210–250
Ob16	+	+	+	+	+	+	+	+	+	+	140–220
Ob17	+	+	+	+	+	+	+	+	+	+	150–200
Ob18	+	+	+	+	+	+	–	–	+	–	120–160
Ob19	+	+	+	+	+	+	+	+	+	+	200–250
Ob20	+	+	+	+	+	+	+	+	+	+	125–210
Ob21	+	+	–	+	+	+	+	–	–	+	220–260
Ob22	+	+	+	+	+	+	–	+	+	–	125–230
Ob23	+	+	–	–	+	+	–	–	–	+	140–210

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