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ORIGINAL ARTICLE

Uncoupling of sexual reproduction from homologous recombination in homozygous *Oenothera* species

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Salient features of the first meiotic division are independent segregation of chromosomes and homologous recombination (HR). In no sexually reproducing, homozygous species studied to date HR is absent. In this study, we constructed the first linkage maps of homozygous, bivalent-forming *Oenothera* species and provide evidence that HR was exclusively confined to the chromosome ends of all linkage groups in our population. Co-segregation of complementary DNA-based markers with the major group of AFLP markers indicates that HR has only a minor role in generating genetic diversity of this taxon despite its efficient adaptation capability. Uneven chromosome condensation during meiosis in *Oenothera* may account for restriction of HR. The use of plants with ancient chromosomal arm arrangement demonstrates that limitation of HR occurred before and independent from species hybridizations and reciprocal translocations of chromosome arms—a phenomenon, which is widespread in the genus. We propose that consecutive loss of HR favored the evolution of reciprocal translocations, beneficial superlinkage groups and ultimately permanent translocation heterozygosity.
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Introduction

Why sexual reproduction should be virtually ubiquitous in eukaryotes is one of the most intriguing puzzles in evolutionary biology (Otto and Lenormand, 2002; de Visser and Elena, 2007). It is generally thought that the long-term advantage of sexual recombination lies in the coupling of beneficial mutations and the elimination of deleterious mutations thereby, preventing degeneration of chromosomes. Thus, homologous recombination (HR) should promote genetic variance in fitness and facilitate an efficient adaptation to changing environments. With rare exceptions and in contrast to an asexual life style members of sexually reproducing populations need to produce females and males, whereas only the first is capable of giving birth to the progeny. In competition with same-gender individuals both have to find each other to ensure fertilization. Therefore, evolutionary driving forces responsible for the origin and maintenance of sex and HR are still enigmatic particularly with regard to the disadvantage of this 'twofold' cost of producing

males (Smith, 1978; West *et al.*, 1999; Rice and Chippindale 2001; Butlin, 2002; Rice, 2002; Nielsen, 2006; Otto and Gerstein, 2006).

In no bivalent forming, sexually reproducing species studied to date HR is absent in both sexes (Hadany and Comeron 2008). However, the frequency of HR can vary among chromosomes and chromosomal regions, between sexes, and in species that differ in life history or ecology (Butlin, 2005; Agrawal, 2006; Wilfert *et al.*, 2007). HR seems central to meiosis because it ensures both interchromosomal recombination between homologous pairs, rather than sister chromatids, and accurate segregation of the entire chromosome set. Thus, HR could also be regarded as an incidental consequence of crossing over required for stabilizing pairing and for avoiding aneuploidy (Cavalier-Smith, 2002; Wijnker and de Jong, 2008). The genus *Oenothera* (evening primrose) provides a model system for investigating these issues. It represents a well-known group of flowering plants, for which a rich source of taxonomic, biosystematic and genetic information is available (Cleland, 1972; Harte, 1994; Dietrich *et al.*, 1997; Levin, 2002; Mráček *et al.*, 2006; Golczyk *et al.*, 2008; Rauwolf *et al.* 2008; Greiner *et al.*, 2008a; Johnson *et al.*, 2010). Furthermore, *Oenothera* is an interesting genus in which many species are essentially sexual clones (Rauwolf *et al.*, 2008; Johnson *et al.*, 2010). They reproduce sexually via seeds but free segregation of chromosomes and HR is restricted in permanent trans-

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location heterozygote (PTH) species (Cleland, 1972; Holsinger and Ellstrand, 1984; Harte, 1994). In PTH species chromosome arms 1–14 instead of chromosomes I–VII are counted because reciprocal translocations of all chromosome arms encompass the entire chromosome complement. Therefore, the genotype designation in *Oenothera* is based on the disposition of chromosome arms of two haploid chromosome sets, so called Renner complexes, for example, *O. biennis* strain suaveolens Grado consists of the two Renner complexes ^{G}albicans and ^{G}flavens with the chromosome arm arrangement $1 \cdot 12 \ 3 \cdot 6 \ 5 \cdot 7 \ 9 \cdot 4 \ 10 \cdot 11 \ 13 \cdot 8 \ 2 \cdot 14$ and $1 \cdot 4 \ 3 \cdot 2 \ 5 \cdot 6 \ 7 \cdot 10 \ 9 \cdot 8 \ 11 \cdot 12 \ 13 \cdot 14$, respectively (Cleland, 1972; Harte, 1994; Rauwolf *et al*., 2008). In consequence, in the two Renner complexes of PTH species no chromosome is fully homologous to any other and no bivalents are formed. Instead, all chromosomes are arranged end to end in a ring during diakinesis and the parental chromosomes are oriented in an alternate order (Cleland, 1936, 1972; Burnham, 1962; Golczyk *et al*., 2005 and 2008). Self-incompatibility, selective fertilization, gamtetophytic and/or zygotic lethal systems often lead to sex linkage of two different chromosome complexes—typically one passes through the pollen and the other through the egg cell, which maintains heterozygote advantages (Cleland, 1972; Rauwolf *et al*., 2008). Not only in *Oenothera*, but also in about a dozen other plant species—mostly in Onagaceae species—reciprocal translocation of chromosome arms is known (Holsinger and Ellstrand, 1984; Levin, 2002; Golczyk *et al*., 2005).

Oenothera is a classical model for suppression of HR in PTH species. Mechanistically, two contradictory models for this phenomenon are found. As originally proposed by Darlington, (1931) break points supporting reciprocal translocations of chromosomes arms in *Oenothera* can be elsewhere in a chromosome than the centromere. As a result, interchanged chromosome regions are homologous for their terminal segment but not for their proximal part. In consequence, meiotic paring and crossing over in PTH species is restricted to the homologous, terminal, (sub)telomeric regions, and suppressed in the non-homologous proximal part. It has therefore long been assumed that HR is repressed in ring-forming PTH species but not necessarily in homozygous ones, not involved in interchanges. This concept is widely accepted throughout the literature (refer Stebbins, 1950; Stebbins, 1971; Levin, 2002; Ranganath, 2008; Johnson *et al*., 2009a, 2010; Johnson, 2011). Cleland, (1972) introduced another view on suppression of HR in *Oenothera*. He assumed that the breaking point of chromosomes involved in arm interchanges occurs within the heterochromatic centromeres. This should still allow recombination in ring-formers—interchanged chromosomes arms are still fully homologues and could potentially pair over their entire length. According to this hypothesis the observed suppression of HR in PTH is mediated by uneven condensation of chromosomes and limitation of chiasmata formation to the telomeric regions. The situation for bivalent formers remained unclear so far because available genetic data are insufficient to allow definitive conclusions and studies using lines with untranslocated chromosomes arms in original species are missing (Cleland, 1972; Harte, 1994).

To test these both models, we generated the first genetic maps of *Oenothera* species using amplified fragment length polymorphism (AFLP) and derived complementary DNA markers (Bensch and Akesson, 2005, Meudt and Clarke, 2007). We show for the first time that even in bivalent-forming, homozygous *Oenothera* species, HR is absent on the majority of all chromosomes in both sexes and restricted to the very distal chromosomal regions. The material used in this study displays the so-called 'Johansen' arm arrangement of chromosomes ($1 \cdot 2 \ 3 \cdot 4 \ 5 \cdot 6 \ 7 \cdot 10 \ 9 \cdot 8 \ 11 \cdot 12 \ 13 \cdot 14$) and is considered to be the most ancient arrangement of the entire population of the subgenus *Oenothera*. It is widely distributed in North America, present in different open pollinating species, and free of lethal factors (Cleland, 1972). Furthermore, bivalent-forming chromosomes in partial complex heterozygous species were generally not involved in interchanges of chromosomes and often represent unaltered, original chromosomes (Cleland, 1972). As we used homozygous species with this ancient chromosomal arrangement they likely harbor unaltered 'original' chromosomes never involved in interchanges (Cleland, 1972), we suggest that restriction of HR occurred before evolution of reciprocal translocation and independent of hybridization events. We propose that restriction of HR in homozygous species favored the evolution of reciprocal translocations and ultimately permanent translocation heterozygosity by rearranging single chromosomes to superlinkage groups allowing co-segregation of beneficial mutations.

Materials and methods

Plant material
Details of genetic constitution and corresponding references for *Oenothera* strains used in this work, as well as field growth and crossing experiments were described elsewhere (Rauwolf *et al*., 2008). An overview about strains used in this work is given in the Table 1. We first chose to genotype the F2 generation obtained from an interspecific cross of the inbred and homozygous parental plants *O. grandiflora* strain grandiflora Tuscaloosa III^{tusca} (female genotype: BB or ^{h}tuscaloosa $\cdot$ ^{h}tuscaloosa) and *O. elata* subsp. *hookeri* strain johansen Standard III^{lamS} (male genotype: A_1A_1 or ^{h}johansen Standard $\cdot$ ^{h}johansen Standard). The two diploid lines share the same ancient chromosome arrangement ($1 \cdot 2 \ 3 \cdot 4 \ 5 \cdot 6 \ 7 \cdot 10 \ 9 \cdot 8 \ 11 \cdot 12 \ 13 \cdot 14$) and carry plastome type III, which ensures regular segregation of bivalent-forming chromosomes and fully fertile plastome-genome constitutions in the progeny, respectively (Rauwolf *et al*., 2008). To refine our data we secondly selected two homozygous subspecies poor of the A genotype with identical chromosome arrangement ($1 \cdot 4 \ 3 \cdot 2 \ 5 \cdot 9 \ 7 \cdot 10 \ 6 \cdot 8 \ 11 \cdot 12 \ 13 \cdot 14$), both originating from the same habitat in Mexico (Steiner, 1955). *O. elata* subsp. *elata* strain elata Cholula (A_2A_2) served as the maternal parent and strain elata Puebla (A_3A_3) as the pollen donor.

AFLP analysis
Isolation of genomic DNA, design of AFLP reactions and DNA fragment detection were performed essentially as reported recently (Rauwolf *et al*., 2008). The primer combinations yielding the highest number of polymorphic bands for genotyping are listed in (Supplementary Tables S3-S8). AFLP markers were designated as described (Peters *et al*., 2001). Co-dominant expression

Table 1 *Oenothera* strains used in this work

Species or hybrid	Strain[a]	Basic genotype[b]	Renner complex[c]	Segmental arrangement[d]	Diakinesis	Relevant reference
Oe. elata subsp. *elata*	elata Cholula	A₂A₂-I	[h]cholula	1·4 3·2 5·9 7·10 6·8 11·12 13·14	7 pairs	Steiner, 1955
			[h]choulua	1·4 3·2 5·9 7·10 6·8 11·12 13·14		
Oe. elata subsp. *elata*	elata Puebla	A₃A₃-I	[h]puebla	1·4 3·2 5·9 7·10 6·8 11·12 13·14	7 pairs	Steiner, 1955
			[h]puebla	1·4 3·2 5·9 7·10 6·8 11·12 13·14		
Oe. grandiflora	grandiflora Tuscaloosa	BB-III	[h]tuscaloosa	1·2 3·4 5·6 7·10 9·8 11·12 13·14	7 pairs	Steiner and Stubbe, 1984
			[h]tuscaloosa	1·2 3·4 5·6 7·10 9·8 11·12 13·14		
Oe. elata subsp. *hookeri* x *Oe. glazioviana*[e]	johansen Standard III[lamS]	A₁A₁-III	[h]johansen Standard	1·2 3·4 5·6 7·10 9·8 11·12 13·14	7 pairs	Rauwolf *et al*., 2008
			[h]johansen Standard	1·2 3·4 5·6 7·10 9·8 11·12 13·14		

For detailed taxonomy of the section *Oenothera* see Dietrich *et al*., 1997.
[a]The first name of the strains refers to the species, which were originally described in the literature, the second one to the collection site and/or the genetic name of a strain.
[b]Latin capital letters refer to the basic nuclear genotype and roman numbers to the basic plastome genotype. For details see Cleland (1972) and Harte (1994).
[c]The subscript 'h' refers to haplo- or haploid-complex. This designation is used throughout the literature for lethal factor-free Renner complexes.
[d]Segmental arrangemts follow the Cleland system.
[e]The strain is a hybrid of the nuclear genome of *Oe. elata* subsp. *hookeri* strain johansen Standard (Cleland, 1935) (*Syn*: hookeri Johansen, Johansen, johansen) and the chloroplast genomes of *Oe. glazioviana* strain rr-lamarckiana Sweden (Heribert-Nilsson, 1912). For details see Rauwolf *et al*., 2008.

sequence tag-derived markers were designated as described (Mráček *et al*., 2006; Rauwolf *et al*., 2008).

Statistical analysis

LOD scores and genetic linkage maps using the Kosambi function were calculated using the JoinMap program (Kyazma BV, Wageningen, The Netherlands). GeneScan analysis and Genotyper software (Applied Biosystems Inc, Lincoln, NE, USA) were used to capture genotyping and segregation data for each polymorphic marker. Polymorphic bands were selected with 0.5-bp tolerances. The data were stored in the Access database (Microsoft, Redmond, WA, USA).

Cytological analysis

The configuration of meiotic chromosomes was determined from inflorescences of appropriate age essentially as described (Golczyk *et al*., 2008; Rauwolf *et al*., 2008). To depict meiotic configurations in a single focal plane, frames were captured, stacked and combined using Combine ZM software (http://www.hadleyweb.pwp. blueyonder.co.uk).

Results

Reduction of HR in homozygous *Oenothera* species

The frequency of HR between markers can be utilised to establish genetic maps. This approach has been used in this study to construct the first molecular linkage maps of the diploid *Oenothera* strains *O. elata* subsp. *hookeri* strain johansen Standard III[lamS] (A₁ genome) and *O. grandiflora* strain grandiflora Tuscaloosa III[tusca] (B genome).

Of 10 245 AFLP fragments obtained using 120 primer combinations, 39.8% displayed polymorphism between the parental lines, which is within the range observed in other interspecific mapping populations (Supplementary Table S1). First, maps with sex-specific markers were generated based on 80 meioses and 1582 polymorphic markers. These fell into seven linkage groups congruent with the seven chromosomes of *Oenothera* genomes (Table 2) (Supplementary Table S2). Surprisingly, most markers in each linkage group (93.3% on average) strictly co-segregated (Table 2) (Supplementary Figure S1). The population size was therefore increased to 244 plants, and 222 markers were used to obtain reliable estimates of recombination frequencies. Segregation distortion in several linkage groups (Table 2) indicated some inbreeding depression and/or epistatic interactions (Grant-Downton and Dickinson, 2004). But, remarkably, among 3,416 meiotic chromosomes examined, an average of 88.5% of the markers did not recombine even once and were again grouped in single clusters (Figure 1, Table 2). Furthermore, 93.2% of additionally integrated, expression sequence tag-derived, co-dominant markers also failed to recombine and clustered in the same groups, allowing determination of the cluster genotypes and merging of the two maps (Figure 2) (Supplementary Figure S1). The major marker groups were flanked by only a few, very distally localized, rarely recombining markers in all chromosomes. The small fraction of recombining markers and their surprisingly low recombination frequency (0.4–7.8%) severely limit the genetic resolution of the map (Figures 1 and 2).

Thus, on the basis of co-segregation of expression sequence tag-derived markers with the major groups of AFLP markers and the cytological data (see below), we suppose that the recombining, short terminal chromosomal regions are gene-poor and that HR has only a minor role in generating genetic diversity even in homozygous species. Moreover, some terminal AFLP markers that do show recombination tended to form small subclusters comprising up to seven loci (Supplementary Figure S1), reflecting the presence of preferential sites of recombination and ensuring exchanges of presumably co-evolved alleles located on defined chromosomal segments.

Reduction of HR is independent of sequence divergence

To exclude the possibility that the degree of sequence divergence between pairing homologous chromosomes

Table 2 Numbers of recombining and non-recombining molecular markers and segregation ratios of all linkage groups calculated from F2 plants of [h]johansen Standard/[h]tuscaloosa (A_1B) hybrids

Linkage groups	Number of A_1 markers based on 80 meioses		Number of B markers based on 80 meioses		Number of integrated markers based on 488 meioses		Segregation ratios of single linkage groups
	Clustered	Recombining	Clustered	Recombining	Clustered	Recombining	$A_1A_1{:}A_1B{:}BB$
1	124	8	137	3	42	4	0.4:2.1:1.5
2	115	16	125	9	29	8	0.8:2:1.2
3	101	9	98	2	31	3	1.2:2.0:0.8
4	114	11	99	14	30	5	0.9:2.1:1
5	84	4	75	19	20	4	1.1:2:0.9
6	103	2	111	4	23	2	0.6 1.8:1.6
7[a]	104	5	126	4	26	2	0.8:2.2:1
Sum	745	55	771	55	201	28	
Percentage	93.1	6.9	93.3	6.7	87.8	12.2	

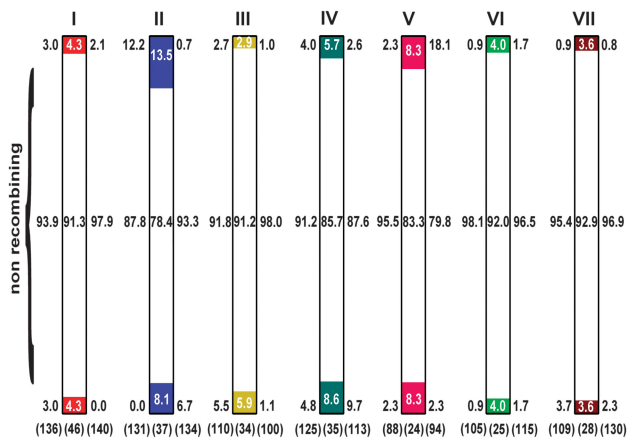[a]Chromsome 9·8, see Supplementary Figure S1 and Rauwolf *et al.*, 2008.

Figure 1 Schematic view of recombining regions of all seven *Oenothera* chromosomes. Non-recombining (white) and recombining (color) chromosomal regions are located in large proximal and much shorter, far distal regions, respectively. The lengths of the regions correspond to the relative numbers of markers per chromosome. The relative numbers of markers specific for [h]johansen Standard (A_1) and [h]tuscaloosa (B), indicated to the left and right of chromosomes, respectively, are based on the analysis of 80 meioses. The relative numbers of markers merged in the integrated map are based on 488 meioses and indicated within the ideograms. The total numbers of markers used in this study are indicated in parentheses. Because of the low genetic resolution of the maps the sizes of the chromosomes were normalized

of the basic A_1 and B genomes is responsible for lack of recombination, a second F2 linkage analysis was performed using an intraspecific cross. For this purpose we chose to genotype the F2 generation of the inbred and homozygous parental plants with the genotypes A_2A_2 and A_3A_3 (see Materials and methods section). Overall, 10.9% of the bands detected were polymorphic that is four times less than the value for the interspecific cross and reflects the close relationship between the two strains. Again, only a small portion of 6.5% of investigated markers recombined, based on the analysis of 560 meiotic chromosomes (Supplementary Table S1).

Unusual chromosome behavior during meiosis
Cytological studies were performed to elucidate whether reduction of recombination is accompanied by alterations in chromosome behavior during meiosis (Figures 3a and d). The data of both A_1 and B parental species (data not shown) and their F1 hybrids consistently confirmed pairing of seven homologs at diakinesis and regular meiotic segregation at late anaphase I (Figures 3c and d). During the remaining stages the chromosome behavior was quite unusual. Briefly, in contrast to the usual pattern, all unpaired 14 chromocenters, which represent large pericentromeric segments (Figure 3a) cluster in early meiotic prophase and remain clustered until late pachytene within a highly polarized Rabl configuration (Figure 3b). The observed exceptional uneven chromosome condensation in homozygous A_1B hybrids seems to be characteristic for all *Oenothera* species studied so far (Wisniewska, 1935; Kurabayashi *et al.*, 1962; Cleland, 1972; Golczyk *et al.*, 2008). The chromosomes consist of large and highly condensed centromere-proximal segments and display relaxed distal regions during prophase I (Figure 3b). The decondensed distal chromosome portions are becoming smaller as the meiosis progresses (Figures 3b and c). The less condensed distal chromosome segments in the seven bivalents are terminally attached indicating crossing over events (Figure 3c). Only at very late diakinesis all chromosomes finally become evenly compacted throughout their entire length, as we described recently for other *Oenothera* species (Golczyk *et al.*, 2008). This structural peculiarity may account for the lack of HR over almost the entire lengths of all chromosomes in the genus.

Discussion

Collectively, our data uncover the striking fact that HR is repressed over about 90% length of all chromosomes. The observed terminal attachments of chromosomes may be necessary to ensure regular segregation of chromosomes and indicate that crossing over and presumably HR frequently occurs but is exclusively restricted to the very distal, gene-poor, chromosomal regions in homozygous, bivalent-forming *Oenothera* species. Distal recombination events possibly escaped detection analyzing 80 and 488 meiosis with 1,582 and 222 polymorphic markers, respectively, either because of their low frequency or because of their terminal position. At least in ring-forming PTH species it remains possible that the number of recombination at chromosome ends is some-
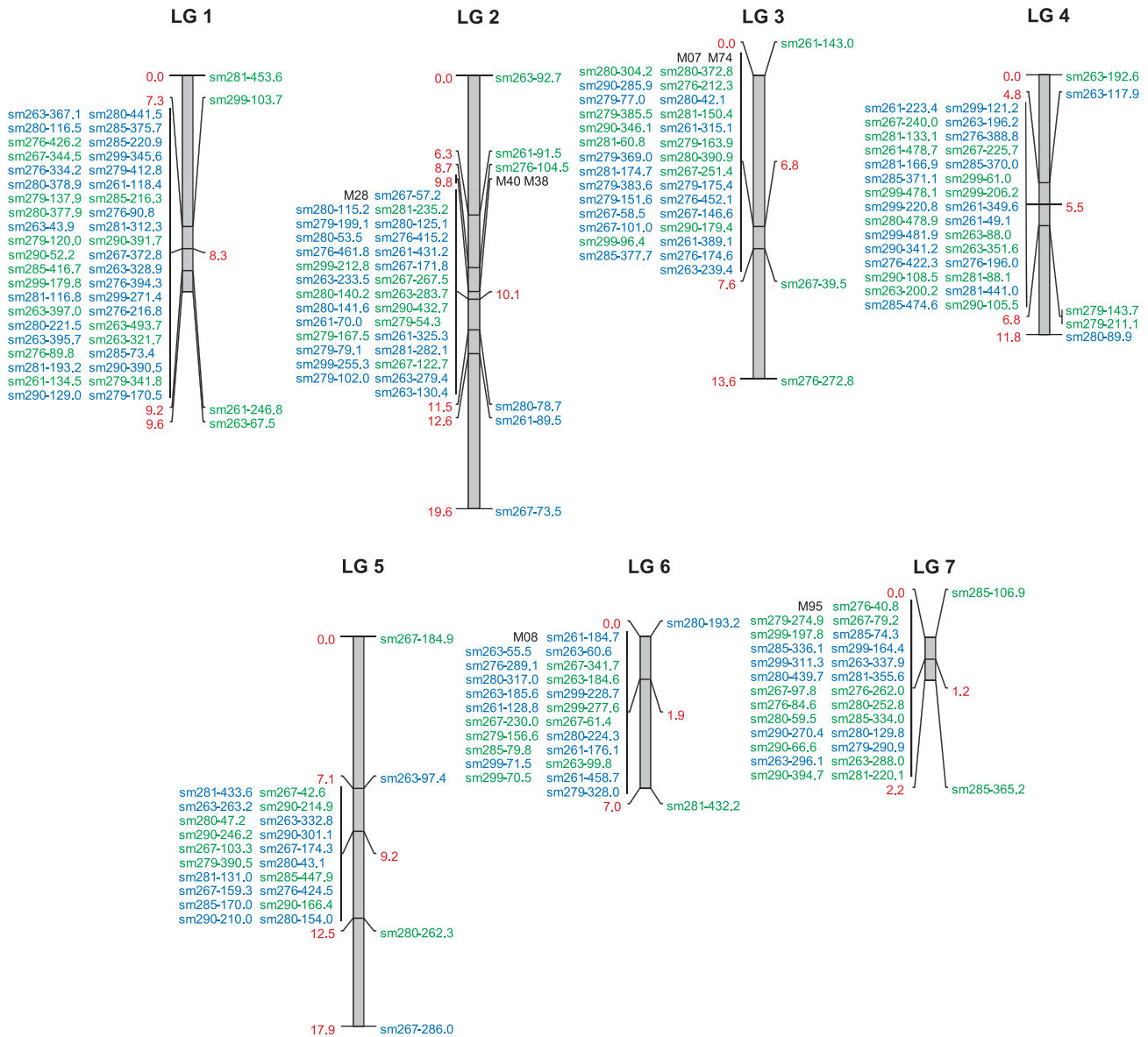
Figure 2 Integrated AFLP map of *Oenothera* species. The molecular maps of ʰjohansen Standard and ʰtuscaloosa are based on the analysis of 488 meioses and 229 molecular markers. Co-dominant markers are marked in black, AFLP markers of ʰjohansen Standard and ʰtuscaloosa are marked in green and blue, respectively. The size of the chromosomes in centimorgan (red) corresponds to the genetic resolution.

what underestimated due to a presumed higher degree of homology presumably pointing to a limited number of polymorphisms in this region (Cleland, 1972; Rauwolf *et al*., 2008). However, the relatively high degree of genetic diversity detected between the parental species *O. elata* subsp. *hooerki* and *O. grandiflora* and predating their divergence about 500,000 years ago (Levy and Levin, 1975; Greiner *et al*., 2008b) argues against an underestimation of HR at chromosome ends in our mapping population.

This is the first case in which sex and HR have been found to be uncoupled over a length of ∼90% of all chromosomes in a bivalent-forming, homozygous and sexually reproducing eukaryotic organism, allowing all chromosomes in both sexes to be maintained essentially unchanged over generations. The only recombination events observed at the chromosome ends suggest

that HR is spatially regulated. As most complementary DNA markers used in this study also cluster in the co-segregated group of most markers, HR is insufficient to provide significant genetic diversity. Nevertheless, the successful cosmopolitan subgenus *Oenothera* demonstrated a remarkably efficient adaptation capability and occupation of new environments in its evolutionary history (Dietrich *et al*., 1997).

So far, the limited number of available phenotypic markers and the appearance of reciprocal translocations, the occurrence of balanced lethals, as well as megaspore, embryo sac and pollen tube competition between distinct chromosomal complexes have previously prevented precise determination of overall rates of recombination in *Oenothera* species (Cleland, 1972; Harte, 1994; Rauwolf *et al*., 2008). Suppression of HR in ring-forming *Oenothera* species was long thought to be
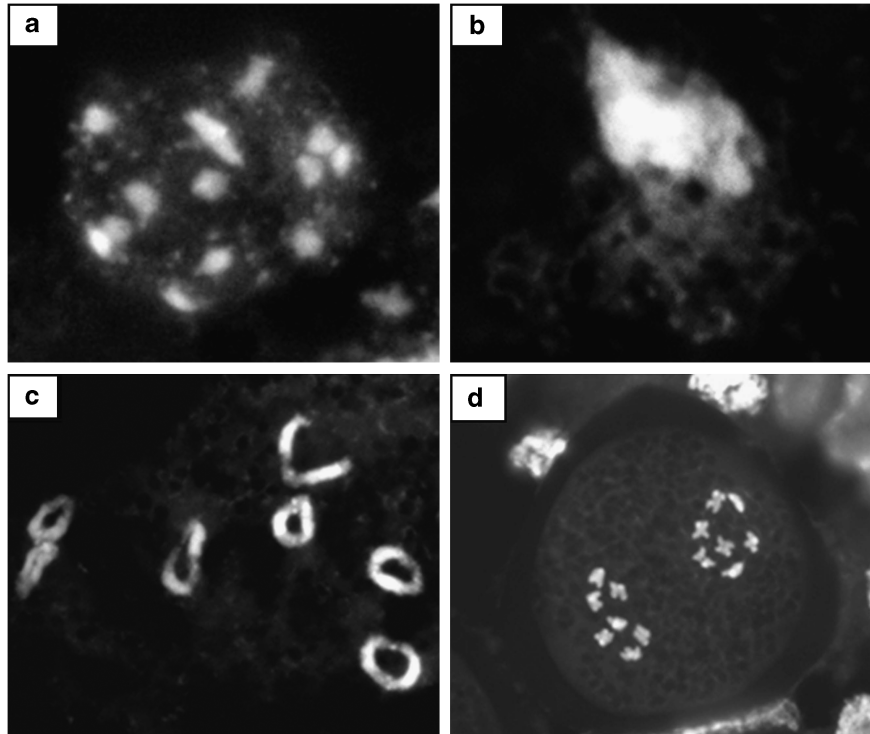
Figure 3 Cytology of pre-meiotic and meiotic phases in ʰjohansen Standard ʰtuscaloosa (A₁B) hybrids. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize DNA. (**a**) Pre-meiotic nuclei with 14 chromocenters. (**b**) Rabl configuration at zygotene, showing a strongly stained condensed pericentromeric pole and decondensed distal chromosome segments. (**c**) Early diakinesis. Less condensed distal chromosome segments in the seven bivalents are terminally attached. (**d**) Late anaphase I. Chromosomes have segregated regularly.

a consequence of reciprocally translocated chromosome arms (Darlington, 1931; Levin, 2002; Ranganath, 2008; Johnson, 2010). Our data clearly disprove this long-standing hypothesis. Furthermore, we could demonstrate that HR is also suppressed in bivalent-forming *Oenothera* species.

Ecological and evolutionary conclusions of recent papers are based on the assumption that HR is normal in homozygous *Oenothera* species as compared with PTH species (Johnson *et al.*, 2009a, 2010). According to our data only free segregation of chromosomes but not HR may account for ecological and reproductive differences between homozygous and complex-heterozygous species.

It was already speculated by Cleland (1972) that a poorly known kinetics of uneven chromatin condensation during meiosis may be responsible for restriction of HR in *Oenothera*. Perhaps, only the less condensed terminal regions at the defined meiotic period might be available for recombination and presumably provide chromosome-specific anchors for pairing in *Oenothera*. Chromatin conformation is a key factor limiting HR in plants (Kirik *et al.*, 2006; Goodstadt and Ponting, 2011). Therefore, uneven condensation is likely to be a major impediment to meiotic recombination not only in complex-heterozygous PTH species, but also in homozygous *Oenothera*. However, we cannot rule out the possibility, that also the supposed ancestral 'Johansen' arrangement in A₁ and B genomes results from a so far unknown complex karyotype evolution, which may have a general influence on HR in the whole genus.

Nevertheless, the data presented here strongly suggest that restriction of HR is not a by-product of the structural consequences of reciprocal translocations, but may have arisen in homozygous, bivalent-forming *Oenothera* species already before its occurrence. This view is substantiated by the fact that most PTH *Oenothera* species derived independently from bivalent-forming ancestors and that the ʰjohansen Standard and ʰtuscaloosa genomes used in this study possess ancestral chromosome arrangements (Cleland, 1972; Dietrich *et al.*, 1997; Levin, 2002).

These findings shed new light on the development of alternative genetic strategies in adaptive evolution. Apparently, a sequence of three distinct modes of coupling the genetic material has conferred coherent evolutionary advantages on *Oenothera*. On the basis of our data we propose that progressive restriction of HR at proximal regions was the first step fixing genetic material to defined chromosomal segments and eventually to entire linkage groups, which were still able to segregate in a Mendelian manner. These original species may have existed as normal open-pollinating diploid species, formed seven bivalents during meiosis and were characterized by median centromers (Cleland, 1972). Because of restriction of pairing and crossing over to the distal segments of the chromosomes tight co-evolution of linked alleles was established. The genetically fixed gene combinations in translocated segments acquired particular selective value favoring stability of translocations (refer Stebbins, 1950). Subsequently, consecutive reciprocal translocations initially stabilized a few, and

ultimately all, chromosomes in a single linkage group. This prevented free segregation of individual chromosomes and, together with general species interfertility, partially compensated for the reduction of HR, while retaining the advantages of heterosis. This is particularly relevant when species switched from outbreeding to autogamy (Johnson *et al.*, 2009b). Finally, hybridization of species, in combination with the acquisition of gametophytic and sporophytic lethals, generated a balanced genetic system as a refinement of a consistent evolutionary scenario. Stable heterozygosity of the nuclear genotype encompassing all chromosomes was ultimately accomplished.

Oenothera is a highly successful genus, which originated in the Americas, reached Europe after 1500 A.D. and has since invaded all continents (Harte, 1994; Dietrich *et al.*, 1997). This demonstrates that it is possible to establish an intermediate position between sexual and asexual modes of life, in which HR is decoupled from sexual reproduction. Furthermore, asexual lineages are known from most major plant and animal groups and their persistence over geological time proves that they retain sufficient capacity to adapt (Welch and Meselson, 2000; Ozias-Akins and van Dijk, 2007). Originally, when sporophytic lethals still did not accumulate in partial and/or permanent complex heterozygotes, the introduction of one or more beneficial foreign chromosomes into a stable genetic system by cross-pollination can lead to a steady improvement of the chromosome set, and potentially represents an effective form of adaptation.

The acquisition of new gene combinations by repeated successful inter- and intraspecific hybridization over generations with regard to individual chromosomes or—more advanced—to entire chromosome sets also contributes to ecological divergence and provides an escape from mutational load.

Furthermore, the PTH state provides the benefit to the species of maintaining seed set as sexual 'clones' with a greater probability of spreading over a larger area versus more typical methods of asexual propagation.

Conclusions

Our data question the long-standing assumption that HR is primarily repressed due to reciprocal translocation of chromosome arms in ring-forming *Oenothera* species. The factors responsible for evolution of reciprocal translocations are still enigmatic. A pre-requisite seemed to be a similar size of chromosomes with heterochromatic centromeres representing potential break points. Translocations at centromeres would also avoid partial deletions in resulting hybrids und maintain the chromosomal morphology and fertility (Cleland, 1972). In the resulting hybrids of often-unrelated complexes heterosis effects and accumulation of deleterious mutations may have favored complex-heterozygous selection. Consecutive repression of HR in *Oenothera* seemed to be restricted in an early evolutionary stage in bivalent formers and is likely to be a pre-requisite for the evolution of reciprocal translocations and superlinkage groups, because it ensured perpetuation of their advantages. Together with interspecific hybridization, these factors provide an alternative strategy for recombining the genetic material to facilitate stacking of beneficial mutations. Our results show that *Oenothera* provides an ideal model for examining hypotheses regarding the evolutionary advantages of sex, as well as of speciation.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Heredity website (http://www.nature.com/hdy)