*Genet. Res., Camb.* (2008), **90**, *pp.* 111–118. © 2008 Cambridge University Press
doi:10.1017/S001667230700897X Printed in the United Kingdom


# 'Junk' DNA and phenotypic evolution in *Silene* section *Siphonomorpha*

THOMAS R. MEAGHER[1]* AND DENISE E. COSTICH[2]
[1] *Centre for Evolution Genes and Genomics, School of Biology, University of St Andrews, St Andrews, Fife KY16 9TH, UK*
[2] *USDA-ARS, Cornell University, 175 Biotechnology Bldg, Ithaca, NY 14853-2703, USA*

(*Received 12 September 2007 and in revised form 24 October 2007*)

**Summary**

One of the long-standing mysteries in genomic evolution is the observation that much of the genome is composed of repetitive DNA, resulting in inter- and intraspecific variation in nuclear DNA content. Our discovery of a negative correlation between nuclear DNA content and flower size in *Silene latifolia* has been supported by our subsequent investigation of changes in DNA content as a correlated response to selection on flower size. Moreover, we have observed a similar trend across a range of related dioecious species in *Silene* sect. *Elisanthe*. Given the presence of sex chromosomes in dioecious *Silene* species, and the tendency of sex chromosomes to accumulate repetitive DNA, it seems plausible that dioecious species undergo genomic evolution in ways that differ from what one might expect in hermaphroditic species. Specifically, we query whether the observed relationship between nuclear DNA content and flower size observed in dioecious *Silene* is a peculiarity of sex chromosome evolution. In the present study we investigated nuclear DNA content and flower size variation in hermaphroditic species of *Silene* sect. *Siphonomorpha*, as close relatives of the dioecious species studied previously. Although the nuclear DNA contents of these species were lower than those for species in sect. *Elisanthe*, there was still significant intra- as well as interspecific variation in nuclear DNA content. Flower size variation was found among species of sect. *Siphonomorpha* for petal claw and petal limb lengths, but not for calyx diameter. This last trait varies extensively in sect. *Elisanthe*, in part due to sex-specific selection. A negative correlation with nuclear DNA content was found across populations for petal limb length, but not for other floral dimensions. We conclude that impacts of nuclear DNA content on phenotypic evolution do manifest themselves in hermaphroditic species, so that the effects observed in sect. *Elisanthe*, and particularly in *S. latifolia*, while perhaps amplified by the genomic impacts of sex chromosomes, are not limited to dioecious taxa.

## 1. Introduction

The evolution of gender polymorphisms in plant populations is characterized by complexity on many levels (Charlesworth, 2006). For example, gender expression is evolutionarily linked to many aspects of floral evolution, including variation in flower size and overall plant life-history. In species that exhibit gender dimorphism, such as dioecy, sex-specific drivers of floral evolution are relatively easy to study in isolation. Indeed, quantitative genetic models have been applied to good effect to understand sex-specific floral evolution leading to sex differences in flower size and other secondary sex characteristics (Meagher, 1999). Given that dioecy is based on very specific genomic effects, such as sex-limited gene expression and in the extreme the evolution of sex chromosome heteromorphism, it is possible that floral evolution in dioecious species is driven by genomic processes that are specific to dioecy. On the other hand, genomic effects underlying floral evolution, and in particular flower size evolution, might be independent of gender expression. In the present paper, we explore whether genomic effects that influence flower size in dioecious species are also manifested in closely related hermaphroditic species.

* Corresponding author. e-mail: trm3@st-and.ac.uk

One of the long-standing mysteries in genomic evolution is the observation that much of the genome is composed of repetitive DNA in the form of transposable elements and other forms of repetitive sequences (Flavell *et al*., 1983; Heslop-Harrison, 2000; Kidwell & Lisch, 2001; Meagher & Vassiliadis, 2005). Such repetitive DNA has typically been regarded as superfluous to the function of the genome in generating phenotypes, and the introduction of repetitive sequences has often been interpreted as a deleterious effect (Charlesworth *et al*., 1994). More recently, our continuing investigation of the relationship between variation in repetitive DNA, manifested as variation in nuclear DNA content and flower size, suggests that repetitive DNA may play an effective role in adaptive evolution (Meagher *et al*., 2005).

The discovery of a negative correlation between nuclear DNA content and flower size in *Silene latifolia* (Meagher & Costich, 1994) has been supported by investigation of changes in DNA content as a correlated response to selection on flower size (Meagher & Costich, 1996), and the effect has been observed across a range of related species in *Silene* sect. *Elisanthe* (Meagher & Costich, 2004). This work has intriguing implications for the relationship between DNA content variation and phenotypic evolution. We attribute the observed relationship to indirect impacts of repetitive DNA on patterns of gene expression. For example, tandem repetitive DNA is known to influence local protein-DNA interactions; and dispersed repetitive DNA, such as transposable elements, is known to affect gene expression through insertion into regulatory regions of genes. On the basis of our observations, we have proposed that repetitive DNA underlying DNA content variation is a major driver in quantitative phenotypic variation (Meagher & Costich, 1996; Meagher & Vassiliadis, 2005).

A consistent feature of species of *Silene* sect. *Elisanthe* is that they are all dioecious, and there is a well-developed model of sex chromosome evolution within this group (Ainsworth, 1999; Charlesworth, 2002; Westergaard, 1958). Indeed, *S. latifolia* was one of the first known examples of the XX (female) and XY (male) mode of sex determination (Blackburn, 1923, and Winge, 1923, cited in Lengerova *et al*., 2003). This species has also been an important object of study in the development of evolutionary models of Y chromosome evolution, in which the Y chromosome is predicted to accumulate non-coding repetitive DNA sequences (Nicolas *et al*., 2005).

Given the presence of sex chromosomes in dioecious *Silene* species, and the tendency of sex chromosomes to accumulate repetitive DNA, it seems plausible that dioecious species undergo genomic evolution in ways that differ from what one might expect in hermaphroditic species. For example, one might speculate that accumulation of transposable elements in the non-combining region of Y chromosomes could provide a reservoir that could then elevate probabilities of transposable element establishment to other parts of the genome, such as the autosomes, through lateral transmission. Thus, we query whether the observed relationship between nuclear DNA content and flower size observed in dioecious *Silene* is a peculiarity of sex chromosome evolution. More specifically, would a negative correlation between flower size and nuclear DNA content appear in the absence of dioecy?

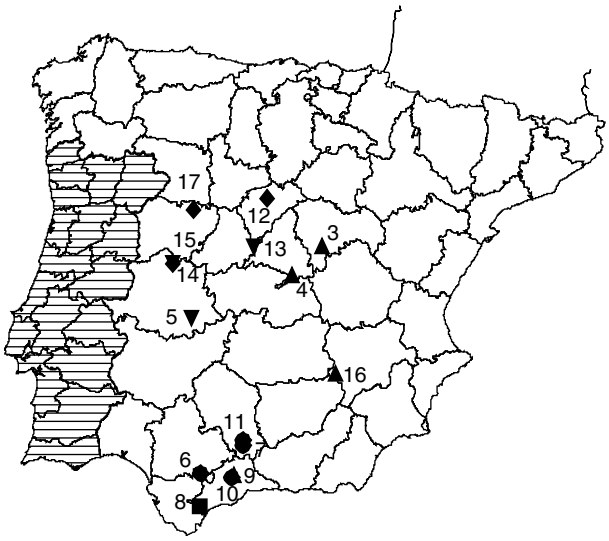


Fig. 1. Map of Spain showing locations of the sampled populations of *Silene* sect. *Siphonomorpha* (circles, *S. andryalifolia*; diamonds, *S. coutinhoi*; squares, *S. fernandezii*; triangles, *S. mellifera*; inverted triangles, *S. nutans*). Precise coordinates for each locality are given in Table 1.

The present study investigated nuclear DNA content and flower size variation in *Silene* sect. *Siphonomorpha*, a group comprised of hermaphrodite species. We addressed the following questions: What is the extent of nuclear DNA content and flower size variation among sect. *Siphonomorpha* species? Does the negative correlation between nuclear DNA content and flower size previously observed in dioecious species of *Silene* hold up for hermaphroditic species? What insights into genome evolution are suggested by the pattern of distribution of nuclear DNA content across species of sect. *Siphonomorpha* (hermaphroditic) in contrast to sect. *Elisanthe* (dioecious)?

## 2. Materials and methods

This study focuses on five species in *Silene* sect. *Siphonomorpha*: *S. andryalifolia*, *S. coutinhoi*, *S. fernandezii*, *S. mellifera* and *S. nutans*. These species are native to the Iberian peninsula, though *S. nutans* is more widespread (Talavera, 1990). In May–June of 1998, populations of each species were located in Spain (Fig. 1, Table 1); floral measurements (calyx

https://doi.org/10.1017/S001667230700897X Published online by Cambridge University Press

Table 1. *Collection localities for* Silene *sect*. Siphonomorpha *populations sampled*

| *Silene* sp. | Site name | Map no. | Longitude | Latitude | $N_{field}$ | $N_{gh}$ |
|---|---|---|---|---|---|---|
| *S. andryalifolia* | Peñon de Algámitas, Sevilla | 6 | −5·18 | 37·01 | 25 | 30 |
| *S. andryalifolia* | Ermita de Pruna, Sevilla | 7 | −4·32 | 37·46 | 25 | 19 |
| *S. andryalifolia* | El Torcal de Antequera, Malaga | 10 | −4·55 | 36·95 | 25 | 29 |
| *S. andryalifolia* | Zuheros, Cordoba | 11 | −4·31 | 37·54 | 25 | 32 |
| *S. coutinhoi* | Ermita de San Frutos, Segovia | 12 | −3·87 | 41·33 | 25 | 25 |
| *S. coutinhoi* | Hervás, Cáceres | 14 | −5·87 | 40·27 | 25 | 29 |
| *S. coutinhoi* | La Orbada, Salamanca | 17 | −5·47 | 41·13 | 25 | 22 |
| *S. fernandezii* | Penas Blancas, Malaga | 8 | −5·18 | 36·49 | 15 | 33 |
| *S. mellifera* | Rio Tajuña, Madrid | 3 | −2·70 | 40·59 | 25 | 21 |
| *S. mellifera* | Belmonte de Tajo, Madrid | 4 | −3·34 | 40·13 | 25 | 25 |
| *S. mellifera* | El Torcal de Antequera, Malaga | 9 | −4·51 | 36·98 | 25 | 29 |
| *S. mellifera* | Puerto del Barrancazo, Albacete | 16 | −2·42 | 38·58 | 25 | 8 |
| *S. nutans* | Puerto de Berzocana, Cacares | 5 | −5·45 | 39·42 | 12 | 18 |
| *S. nutans* | Silla de Felipe II, Madrid | 13 | −4·15 | 40·57 | 25 | 15 |
| *S. nutans* | Hervás, Cáceres | 15 | −5·87 | 40·27 | 25 | 16 |

Map reference numbers for each population as well as latitude and longitude (in degrees) were those used in Fig. 1. Sample sizes for field-based floral measurements ($N_{field}$) and greenhouse-based DNA content assays ($N_{gh}$) are also shown.

diameter, petal claw length [=calyx length] and petal limb length [=1/2 of corolla diameter]) were taken from up to three flowers from 25 plants per population or as many plants as were present (*N* ranged from 12 to 25; Table 1). Seeds were collected during the last week of July 1998 and stored at room temperature in plastic bags until they were planted in late July 2000 and grown under glass at the University of St Andrews (UK). It was our intention to raise plants in the greenhouse to flowering so as to obtain floral measurements under uniform conditions, but these species are long-lived perennials and flowering in the greenhouse even after 7 years of cultivation remains too sporadic to yield a useful sample size. Consequently, our analyses of flower size variation in this paper are based on the original field measurements.

Flow cytometric assays of nuclear DNA content (Costich *et al*., 1991) were performed on leaf material from greenhouse-raised plants (overall number of plants per population accession in our greenhouse population are shown in Table 1). A concern with flow cytometric assays is that apparent DNA content differences might be due to cytosolic interference from secondary compounds (Price *et al*., 2000). We have tested for such effects in *Silene* by including leaf samples from plants that differ in DNA content in a single preparation, thus exposing nuclei from both plants to the same suite of secondary compounds (Meagher & Costich, 2004). This control procedure provided no evidence of cytosolic impacts on DNA fluorescence. We have recently conducted further tests for cytosolic impacts in *Silene* by investigating the relationship between variation in nuclear DNA fluorescence and variation in the fluorescence of the internal standard chicken red blood cell (CRBC) and again found no evidence of cytosolic effects (Looseley & Meagher, in prep.).

Two assays of nuclear DNA content emphasize different features of the genome (Costich *et al*., 1991). Red fluorescence of propidium iodide (PI-DNA) measures overall DNA content, whereas red fluorescence of PI in the presence of chromomycin (PI+CA3-DNA) provides an AT-biased measure that targets repetitive DNA since repetitive motifs in plants are typically AT-biased (Wang *et al*., 1994). For both assays, estimates of DNA content were obtained by dividing the mean fluorescence of *Silene latifolia* nuclei by the mean for an internal CRBC standard and multiplying by 2·33, the DNA content (in picograms) of CRBC (Arumuganathan & Earle, 1991). To estimate DNA content in the PI+CA3-DNA assay, the multiplier of 2·33 was adjusted by multiplying by the mean fluorescence of CRBC among samples with PI+CA3 and dividing by the mean fluorescence of CRBC among samples with only PI. In comparing the relationship between PI-DNA and PI+CA3-DNA in the present study with previously published results for species of sect. *Elisanthe* (Meagher & Costich, 2004), earlier estimates of PI+CA3-DNA were adjusted in the same manner. Because the DNA content estimates for sect. *Siphonomorpha* and sect. *Elisanthe* were obtained at different times and using different flow cytometers, we did not do a quantitative comparison of the two sets of results, but rather limit our consideration to a qualitative comparison.

All statistical analyses were conducted using SAS version 9.1.3 (SAS Institute, 2004). Population effects were considered as nested within species in analyses of variance. Correlations between nuclear DNA content and floral dimensions were based on population-level

Table 2. *Nested analysis of variance (ANOVA) results testing for differences among species populations*

(a) DNA content

| | PI-DNA | | | | PI + CA3-DNA | | | | (PI-DNA) – (PI + CA3-DNA) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | d.f. | MS | *F*-ratio | *P* | d.f. | MS | *F*-ratio | *P* | d.f. | MS | *F*-ratio | *P* |
| Species | 4 | 3·69 | 16·35[a] | 0·0002 | 4 | 1·46 | 8·35 | <0·0001 | 4 | 0·83 | 3·38 | 0·01 |
| Population [Species] | 10 | 0·23 | 2·20 | 0·018 | 10 | 0·30 | 1·69 | 0·081 | 10 | 0·21 | 0·84 | 0·59 |
| Error | 329 | 0·10 | | | 308 | 0·17 | | | 301 | 0·24 | | |

(b) Floral dimensions

| | Calyx diameter | | | | Petal claw length | | | | Petal limb length | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | d.f. | MS | *F*-ratio | *P* | d.f. | MS | *F*-ratio | *P* | d.f. | MS | *F*-ratio | *P* |
| Species | 4 | 0·23 | 0·35[a] | 0·84 | 4 | 2181·38 | 48·60[a] | <0·0001 | 4 | 96·01 | 9·08[a] | 0·0023 |
| Population [Species] | 10 | 0·67 | 6·53 | <0·0001 | 10 | 44·88 | 18·26 | <0·0001 | 10 | 10·57 | 8·08 | <0·0001 |
| Error | 341 | 0·10 | | | 341 | 2·46 | | | 289 | 1·31 | | |

[a] MS(Population[Species]) used as the error MS.

means across the 15 species by population combinations (listed in Table 1). We used population means because we could not directly measure DNA content on plants in the field and, as noted above, we were not able to measure floral traits on plants in the greenhouse. Our estimate of the correlation between these two sets of traits confounds species- and population-level effects. In principle, it would be desirable to take into account phylogenetic relationships among species in determining the correlation between nuclear DNA content and flower size using a method such as independent contrasts (Felsenstein, 2004). That was not possible in our study because the phylogenetic relationships among these species have not been included to date in modern phylogenetic work on the genus *Silene* (B. Oxelman, pers. comm.) Species-level correlations among floral characters within individual plants were calculated by pooling across populations. Numbers of plants included in measures of floral dimensions in field populations and estimation of DNA content in greenhouse populations are indicated in Table 1.

## 3. Results

There was evidence of DNA content variation both within and among species of sect. *Siphonomorpha* (Table 2a, Fig. 2). PI-DNA showed variation at all levels, suggesting that there is substantial potential for the evolution of DNA content variation overall. PI + CA3-DNA differed among species, but did not show strong variation among populations within species. The difference between these two measures of DNA content variation reflects variation in the repetitive DNA component of the genome. The estimated difference between these two measures showed significant variation among species but not among populations within species.

There was significant variation in flower size both within and among species of sect. *Siphonomorpha* (Table 2b, Fig. 2). Interestingly, calyx diameter, which shows sexual dimorphism in dioecious *Silene* species, especially in *S. latifolia*, and which shows extensive variation within and among species of sect. *Elisanthe*, did not exhibit significant variation among species of sect. *Siphonomorpha*. On the other hand, petal claw and petal limb length varied significantly among as well as within species.

There was a strongly significant correlation between the two measures of DNA content variation (Table 3, Fig. 3a). In terms of relationship to flower size (Table 3, Fig. 2), there was a significant negative correlation between each of the two DNA content measures and petal limb, which is similar to what has been observed previously in dioecious *S. latifolia*. However, there was no evidence of correlation between DNA content and calyx diameter or petal claw length. There was also no significant level of correlation among the three floral dimensions as measured at the among-population level (Table 3), but there was a significant correlation among floral traits within individual plants for each of the five species (Table 4).

There is evidence that the genomes of sect. *Siphonomorpha* contain repetitive DNA to a similar extent to levels found in sect. *Elisanthe*, even though

https://doi.org/10.1017/S001667230700897X Published online by Cambridge University Press

Table 3. *Correlations by population and species between nuclear DNA content and flower measurements for* Silene *sect*. Siphonomorpha

| | PI + CA3-DNA | | Calyx diameter | | Petal claw length | | Petal limb length | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| PI-DNA | 0·85 | <0·0001 | 0·00 | 0·99 | 0·30 | 0·27 | −0·53 | 0·045 |
| PI + CA3-DNA | | | 0·16 | 0·57 | 0·14 | 0·62 | −0·53 | 0·040 |
| Calyx diameter | | | | | −0·16 | 0·58 | −0·02 | 0·94 |
| Petal claw | | | | | | | 0·44 | 0·098 |

The sample size (populations) is $N = 15$ throughout.

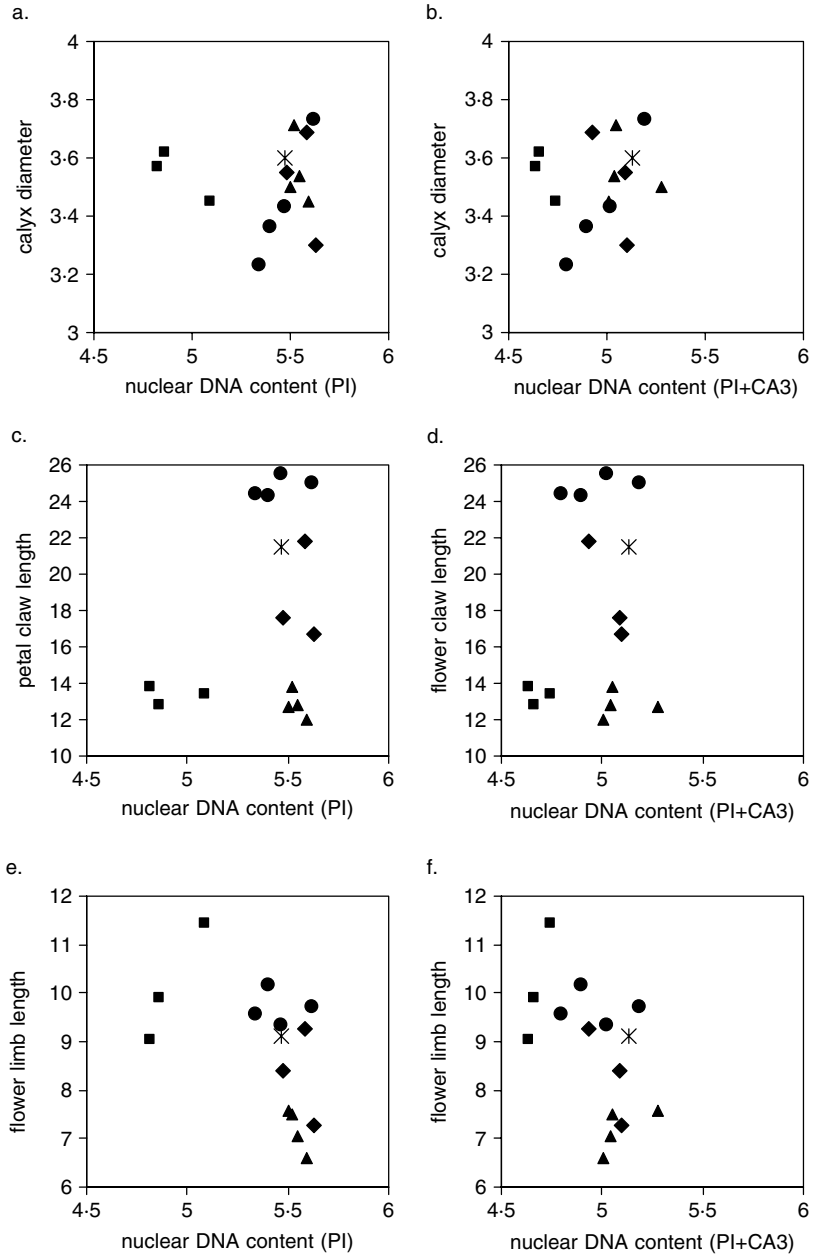


Fig. 2. Relationships between phenotype and PI-DNA or PI + CA3-DNA for calyx diameter (*a*, *b*), petal claw length [= calyx length] (*c*, *d*) and petal limb length (*e*, *f*) for *Silene* sect. *Siphonomorpha* (circles, *S. andryalifolia*; diamonds, *S. coutinhoi*; stars, *S. fernandezii*; triangles, *S. mellifera*; squares, *S. nutans*). DNA content is expressed in picograms, and floral dimensions are expressed in millimetres. Correlation estimates are shown in Table 3.

https://doi.org/10.1017/S001667230700897X Published online by Cambridge University Press

Table 4. *Phenotypic correlations among floral dimensions for species of* Silene *sect*. Siphonomorpha

| Species | | Petal claw length | | | Petal limb length | | |
|---|---|---|---|---|---|---|---|
| | | *N* | *r* | *P* | *N* | *r* | *P* |
| *S. andryalifolia* | Calyx diameter | 100 | 0·21 | 0·040 | 100 | 0·19 | 0·059 |
| | Petal claw | | | | 100 | 0·48 | <0·0001 |
| *S. coutinhoi* | Calyx diameter | 75 | 0·47 | <0·0001 | 37 | 0·40 | 0·015 |
| | Petal claw | | | | 37 | 0·80 | <0·0001 |
| *S. fernandezii* | Calyx diameter | 15 | 0·24 | 0·33 | 15 | 0·12 | 0·61 |
| | Petal claw | | | | 15 | 0·37 | 0·12 |
| *S. mellifera* | Calyx diameter | 100 | 0·32 | 0·0010 | 88 | 0·40 | 0·0001 |
| | Petal claw | | | | 88 | 0·37 | 0·0005 |
| *S. nutans* | Calyx diameter | 62 | 0·10 | 0·43 | 60 | 0·11 | 0·39 |
| | Petal claw | | | | 60 | 0·68 | <0·0001 |

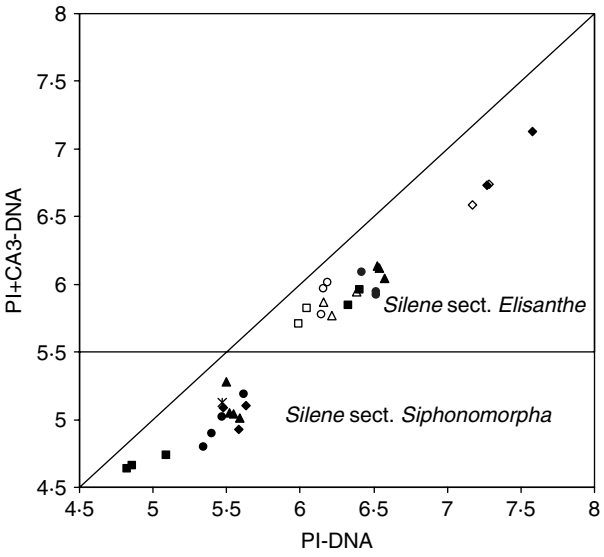


Fig. 3. Impact of CA3 on DNA content estimates for *Silene* sect. *Siphonomorpha* (PI + CA3-DNA < 5·5 pg: circles, *S. andryalifolia*; diamonds, *S. coutinhoi*; stars, *S. fernandezii*; triangles, *S. mellifera*; squares, *S. nutans*) and sect. *Elisanthe* (PI + CA3-DNA > 5·5 pg: circles, *S. latifolia*; diamonds, *S. marizii*; triangles, *S. diclinis*; squares, *S. dioica*; filled symbols, males; open symbols, females). Data for sect. *Elisanthe* are derived from Meagher & Costich (2004).

the genomes of the former are smaller (Fig. 3). This is reflected in the fact that PI + CA3-DNA estimates are lower than PI-DNA estimates across species in both of these sections.

## 4. Discussion

There was considerable intra- as well as interspecific nuclear DNA content variation found in sect. *Siphonomorpha*. This was true for both the PI-DNA and PI + CA3-DNA assays, which suggests that the AT-rich repetitive DNA fraction of the genome follows a similar distribution to that of overall DNA content. Thus, hermaphroditic species of sect. *Siphonomorpha* show variation that is similar in pattern to that found in dioecious species of sect. *Elisanthe*.

There are several different types of repetitive DNA contributing to overall variation (Meagher & Vassiliadis, 2005). The alternative assay used in this study, PI + CA3-DNA, focuses on AT-rich repetitive sequences, which in plants are generally characteristic of tandem repetitive sequences (Wang *et al*., 1994), such as satellite and microsatellite DNA. This category of DNA falls squarely into the historical definition of non-coding 'junk' DNA. However, our previously observed correlation between a flower size measure and PI + CA3-DNA in the dioecious *S. latifolia* suggests that, on the contrary, this class of repetitive DNA has phenotypic impacts. This finding is supported by the negative correlation observed in five hermaphrodite species of sect. *Siphomorpha* in the present study between floral limb length and overall nuclear DNA content (PI-DNA) as well as the AT-biased measure (PI + CA3-DNA). Although tandem repeats are generally not transcribed, and are therefore non-coding in that sense, the proximity of such tandem repeats to expressed genes can moderate their expression (Zuckerkandl, 1997). Thus, accumulation of repetitive sequences may lower expression levels in general, while reduction of repetitive sequences may have the opposite effect. In general, the process of natural selection is not tied to conventional models of allelic substitution, but rather will incorporate whatever features of the genome are capable of being transmitted to the next generation, akin to the phenomenon of 'bricolage' as coined by Jacob (1982; Meagher & Vassiliadis, 2005). Indeed, the role of ancillary non-coding DNA in phenotypic evolution is an area of active investigation (Meagher & Vassiliadis, 2005; Pennisi, 2007; Zuckerkandl, 2002).

https://doi.org/10.1017/S001667230700897X Published online by Cambridge University Press

Another category of repeated sequences that accumulate in the genome is transposable elements that occur as dispersed repeats (McClintock, 1984). These typically have sequence-specific insertion sites such that one can model their evolutionary dynamics in the genome using a population dynamics approach (Charlesworth & Charlesworth, 1983). Transposable elements are considered to be a ubiquitous (or nearly so) feature of eukaryotic genomes (Kidwell & Lisch, 2001). One widespread class of transposons, the LTR-retrotransposons, has been thoroughly characterized (Kumar & Bennetzen, 1999). LTR-retrotransposons consist of a series of genes that code for enzymes involved in their own replication and transmission within the genome, so they are capable of rapid evolutionary change within the genome of a particular lineage. This class of repetitive DNA is also likely to contribute to variation in genome size (Kidwell, 2002) and may play a role in phenotypic evolution as well (Pennisi, 2007). We are presently investigating methods for directly assaying LTR-retrotransposon copy number in *Silene* spp. (Meagher and Yahr, in prep.; Meagher and Loosely, in prep.) in order to determine their potential for contributing to floral evolution in this group.

*Silene* sect. *Siphonomorpha* and sect. *Elisanthe* both showed striking differences between the two DNA assays, suggesting that DNA content variation in the two is strongly driven by AT-rich repetitive DNA. Of course, this is only one of the categories of repetitive DNA likely to contribute to DNA content variation. However, we would expect on theoretical grounds to find more accumulation of certain types of repetitive sequences in dioecious sect. *Elisanthe* species because of the presence of a Y chromosome, which should accumulate repetitive sequences in the non-recombining region (Charlesworth & Charlesworth, 2000; Charlesworth *et al*., 2005). In the case of LTR-retrotransposons, sequences that accumulate on the Y chromosome could also spread through lateral transfer to other chromosomes and become a more widespread genomic feature. Although the overall DNA content of the hermaphroditic species was lower, there was still evidence of considerable intraspecific as well as interspecific variation, with impacts on flower size (petal limb). Even though these species do not have the Y chromosome effects, there are other aspects of their reproductive biology that could contribute to accumulation of repetitive DNA. For example, given the extent of the evolution of morphologically based outbreeding mechanisms in *Silene*, such as gynodioecy and dioecy (Desfeux *et al*., 1996), it is reasonable to assume that hermaphroditic species of *Silene* are self-compatible, and thus potentially selfing. The only species of sect. *Siphonomorpha* that has been studied with regard to its mating system is *S. nutans*, and this species does show evidence of being locally inbred (Van Rossum & Prentice, 2004). Inbreeding is another process that potentially leads to accumulation of repetitive DNA (Charlesworth & Charlesworth, 1995). Further investigation of the mating system in natural populations of sect. *Siphonomorpha*, in conjunction with investigation of the distribution of LTR-retrotransposons and other specific repetitive DNA fractions, would shed further light on the dynamics of repetitive DNA and its role in floral evolution.

In conclusion, intra- and interspecific variation in DNA content, with impacts on phenotypic evolution, as reported in *Silene latifolia*, are not a peculiarity of dioecy, but rather appear to be more widespread. Meagher & Costich (1996) suggested that such effects of repetitive DNA could be an underlying contributor to measures of quantitative genetic variation, as opposed to the conventional model which assumes infinitesimal effects of allelic substitution across multiple coding loci (Bulmer, 1980). The present results suggest that impacts of nuclear DNA content on quantitative variation in flower size is more widespread. Further investigation into the role of repetitive DNA in phenotypic evolution is warranted.

We thank Salvador Talavera for his help in locating field sites and identifying species of sect. *Siphonomorpha*, Laura Meagher for her help in collecting seed, Harry Hodge for his help cultivating plants in the greenhouse and conducting flow cytometry analysis, Bengt Oxelman for his advice on *Silene* systematics, and Daniel Barker for his advice on phylogenetically based comparative methods. We thank P. E. Gibbs, M. E. Looseley and L. R. Meagher for their comments on an earlier draft of this paper. This work was supported by US NSF grant 9726580/0096215 and UK NERC grant NER/T/S/2001/00297.

We dedicate this paper to Deborah Charlesworth, whose contributions to our understanding of the reproductive biology of plants played a critical role in the development of our thinking in this area.

## References

Ainsworth, C. C. (1999). *Sex Determination in Plants*. Oxford: BIOS Scientific Publishers.

Arumuganathan, K. & Earle, E. D. (1991). Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology Reporter* **9**, 217–229.

Bulmer, M. G. (1980). *The Mathematical Theory of Quantitative Genetics*. Oxford: Oxford University Press.

Charlesworth, B. & Charlesworth, D. (1983). The population dynamics of transposable elements. *Genetical Research* **42**, 1–27.

Charlesworth, B. & Charlesworth, D. (2000). The degeneration of Y chromosomes. *Philosophical Transactions of the Royal Society of London, Series B* **355**, 1563–1572.

Charlesworth, B., Sniegowski, P. & Stephan, W. (1994). The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**, 215–220.

Charlesworth, D. (2002). Plant sex determination and sex chromosomes. *Heredity* **88**, 94–101.

Charlesworth, D. (2006). Evolution of plant breeding systems. *Current Biology* **16**, R726–R735.
Charlesworth, D. & Charlesworth, B. (1995). Transposable elements in inbreeding and outbreeding populations. *Genetics* **140**, 415–417.
Charlesworth, D., Charlesworth, B. & Marais, G. (2005). Steps in the evolution of heteromorphic sex chromosomes. *Heredity* **95**, 118–128.
Costich, D. E., Meagher, T. R. & Yurkow, E. J. (1991). A rapid means of sex determination in *Silene latifolia* by use of flow cytometry. *Plant Molecular Biology Reporter* **9**, 359–370.
Desfeux, C., Maurice, S., Henry, J. P., Lejeune, B. & Gouyon, P. H. (1996). Evolution of reproductive systems in the genus *Silene*. *Proceedings of the Royal Society of London, Series B* **263**, 409–414.
Felsenstein, J. (2004). Comparative methods. In *Inferring Phylogenies*, pp. 432–449. Sunderland, MA: Sinauer Associates.
Flavell, R., Jones, J., Lonsdale, D. & O'Dell, M. (1983). Higher plant genome structure and the dynamics of genome evolution. In *Advances in Gene Technology: Molecular Genetics of Plants and Animals*, pp. 47–59. New York: Academic Press.
Heslop-Harrison, J. S. (2000). Comparative genome organization in plants: from sequence and markers to chromatin and chromosomes. *Plant Cell* **12**, 617–635.
Jacob, F. (1982). *The Possible and the Actual*. Seattle: University of Washington Press.
Kidwell, M. G. (2002). Transposable elements and the evolution of genome size in eukaryotes. *Genetica* **115**, 49–63.
Kidwell, M. G. & Lisch, D. R. (2001). Perspective: transposable elements, parasitic DNA, and genome evolution. *Evolution* **55**, 1–24.
Kumar, A. & Bennetzen, J. L. (1999). Plant retrotransposons. *Annual Review of Genetics* **33**, 479–532.
Lengerova, M., Moore, R. C., Grant, S. R. & Vyskot, B. (2003). The sex chromosomes of *Silene latifolia* revisited and revised. *Genetics* **165**, 935–938.
McClintock, B. (1984). The significance of responses of the genome to challenge. *Science* **226**, 792–801.
Meagher, T. R. (1999). Quantitative genetics of sexual dimorphism. In *Gender and Sexual Dimorphism in Flowering Plants* (ed. M. A. Geber, T. E. Dawson & L. F. Delph). New York: Springer.
Meagher, T. R. & Costich, D. E. (1994). Sexual dimorphism in nuclear DNA content and floral morphology in populations of *Silene Latifolia* (Caryophyllaceae). *American Journal of Botany* **81**, 1198–1204.
Meagher, T. R. & Costich, D. E. (1996). Nuclear DNA content and floral evolution in *Silene latifolia*. *Proceedings of the Royal Society of London, Series B* **263**, 1455–1460.
Meagher, T. R. & Costich, D. E. (2004). 'Junk' DNA and long-term phenotypic evolution in *Silene* Section *Elisanthe*. *Proceedings of the Royal Society of London, Series B* (*Biology Letters supplement*) **271**, S493–S497.
Meagher, T. R. & Vassiliadis, C. (2005). Phenotypic impacts of repetitive DNA in flowering plants. *New Phytologist* **168**, 71–80.
Meagher, T. R., Gillies, A. C. M. & Costich, D. E. (2005). Genome size, quantitative genetics and the genomic basis for flower size evolution in *Silene latifolia*. *Annals of Botany* **95**, 247–254.
Nicolas, M., Marais, G., Hykelova, V., Janousek, B., Laporte, V., Vyskot, B., Mouchiroud, D., Negrutiu, I., Charlesworth, D. & Moneger, F. (2005). A gradual process of recombination restriction in the evolutionary history of the sex chromosomes in dioecious plants. *PLoS Biology* **3**, 47–56.
Pennisi, E. (2007). Jumping genes hop into the evolutionary limelight. *Science* **317**, 894–895.
Price, H. J., Hodnett, G. & Johnston, J. S. (2000). Sunflower (*Helianthus annuus*) leaves contain compounds that reduce nuclear propidium iodide fluorescence. *Annals of Botany* **86**, 929–934.
SAS Institute (2004). The SAS system for Windows. Cary, NC: SAS Institute, Inc.
Talavera, S. (1990). *Silene*. In *Flora ibérica: plantas vasculares de la Península Ibérica e Islas Baleares* (ed. S. Castroviejo, M. Laínz, G. López González, P. Montserrat, F. Muñoz Garmendia, J. Paiva & L. Villar). Madrid: Real Jardín Botánico, CSIC.
Van Rossum, F. & Prentice, H. C. (2004). Structure of allozyme variation in Nordic *Silene nutans* (Caryophyllaceae): population size, geographical position and immigration history. *Biological Journal of the Linnean Society* **81**, 357–371.
Wang, Z., Weber, J. L., Zhong, G. & Tanksley, S. D. (1994). Survey of plant short tandem DNA repeats. *Theoretical and Applied Genetics* **88**, 1–6.
Westergaard, M. (1958). The mechanism of sex determination in dioecious flowering plants. *Advances in Genetics* **9**, 217–281.
Zuckerkandl, E. (1997). Junk DNA and sectorial gene repression. *Gene* **205**, 323–343.
Zuckerkandl, E. (2002). Why so many noncoding nucleotides? The eukaryote genome as an epigenetic machine. *Genetica* **115**, 105–129.