



**Supplementary Table S2: Regression model of Total centromere size ~ Genome size × Chromosomes**

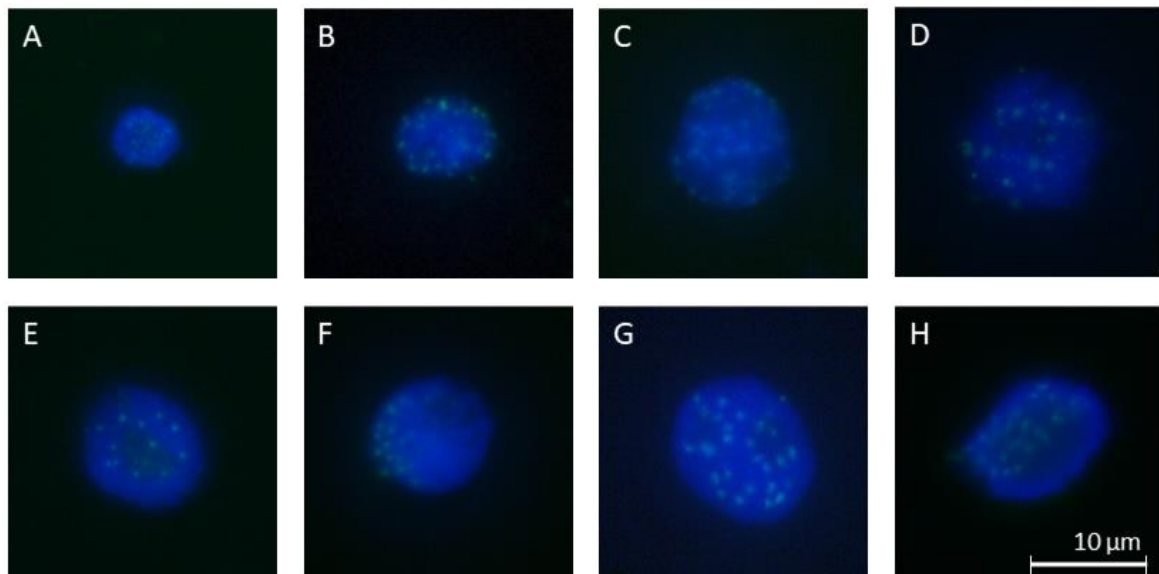
| Model term                                | $b_i$ | $se(b_i)$ | t     | P        |
|-------------------------------------------|-------|-----------|-------|----------|
| Holocentric chromosomes (Intercept)       | 0.404 | 0.355     | -1.14 | 0.257    |
| Genome size                               | 0.952 | 0.121     | 7.86  | 1.61E-12 |
| Metapolycentric chromosomes               | 0.302 | 0.509     | -0.59 | 0.5539   |
| Monocentric chromosomes                   | 0.518 | 0.359     | -1.44 | 0.1515   |
| Genome size × Metapolycentric chromosomes | 0.007 | 0.158     | 0.05  | 0.9638   |
| Genome size × Monocentric chromosomes     | 0.037 | 0.122     | -0.3  | 0.7622   |

Table S2 presents the outcome of multiple linear regression model of the relationship of Total centromere size (log-transformed) to interaction between Genome size (log-transformed) and chromosome type. Note that the interaction model terms are not significant ( $P > 0.05$ ), meaning that the effects of the Genome size on the Total centromere size is independent of chromosome types.

Supplementary Table S1. Measurements of the total contaminant size in 8 grass and 9 gravel species.

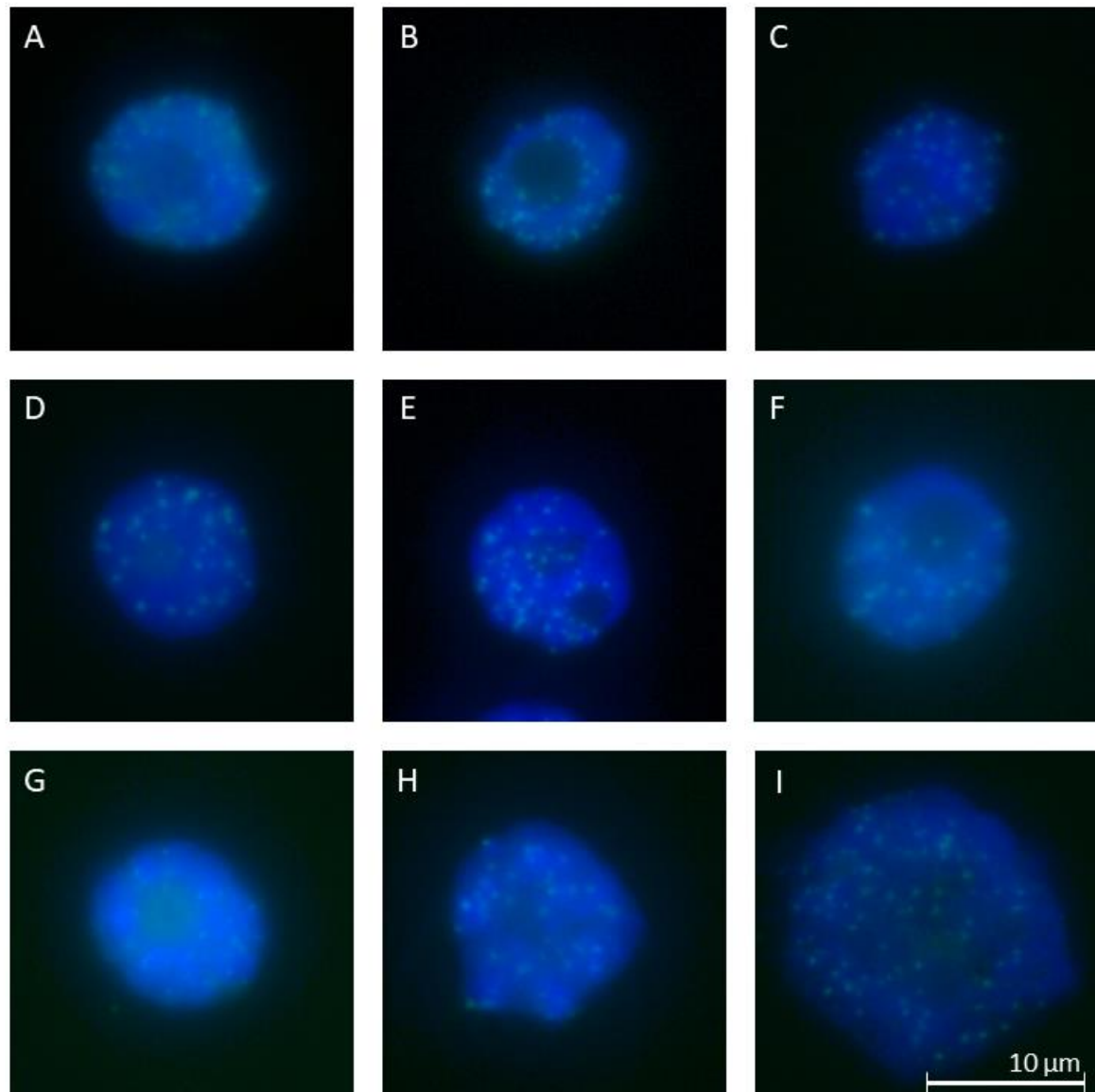
| family    | species                   | code       | sample | nuclei  | DAT_ams[mm2] | O_exposure_time | DAT_ams_Mean | Contid_ams_Mean |
|-----------|---------------------------|------------|--------|---------|--------------|-----------------|--------------|-----------------|
| Festuca   | Medicago arvensis         | MedicAr1   | 1      | 120.877 | 10           | 1.008           | 650          | 1.027           |
| Festuca   | Medicago arvensis         | MedicAr2   | 1      | 138.880 | 10           | 4.398           | 650          |                 |
| Festuca   | Medicago arvensis         | MedicAr3   | 1      | 84.879  | 10           | 5.410           | 850          |                 |
| Festuca   | Medicago arvensis         | MedicAr4   | 2      | 41.844  | 10           | 1.138           | 850          |                 |
| Festuca   | Medicago arvensis         | MedicAr5   | 3      | 50.148  | 10           | 1.138           | 850          |                 |
| Festuca   | Medicago arvensis         | MedicAr6   | 1      | 71.741  | 10           | 4.140           | 650          |                 |
| Festuca   | Medicago arvensis         | MedicAr7   | 2      | 64.145  | 10           | 2.138           | 650          |                 |
| Festuca   | Medicago arvensis         | MedicAr8   | 1      | 81.848  | 10           | 4.140           | 650          |                 |
| Festuca   | Medicago arvensis         | MedicAr9   | 1      | 89.892  | 10           | 5.148           | 500          |                 |
| Festuca   | Medicago arvensis         | MedicAr10  | 1      | 50.178  | 10           | 2.138           | 650          |                 |
| Festuca   | Medicago arvensis         | MedicAr11  | 1      | 57.427  | 10           | 2.708           | 850          |                 |
| Festuca   | Medicago arvensis         | MedicAr12  | 1      | 121.891 | 10           | 2.171           | 500          |                 |
| Festuca   | Medicago arvensis         | MedicAr13  | 1      | 93.897  | 10           | 2.823           | 700          |                 |
| Festuca   | Phlegethon australis      | PhleAr1    | 1      | 41.644  | 10           | 4.094           | 650          | 29.467          |
| Festuca   | Phlegethon australis      | PhleAr2    | 1      | 41.311  | 10           | 6.265           | 650          |                 |
| Festuca   | Phlegethon australis      | PhleAr3    | 1      | 13.384  | 10           | 4.136           | 650          |                 |
| Festuca   | Phlegethon australis      | PhleAr4    | 1      | 42.739  | 20           | 8.038           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr5    | 1      | 30.742  | 10           | 4.140           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr6    | 1      | 22.872  | 20           | 3.934           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr7    | 2      | 23.872  | 20           | 2.185           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr8    | 1      | 21.520  | 10           | 2.922           | 650          |                 |
| Festuca   | Phlegethon australis      | PhleAr9    | 1      | 20.493  | 10           | 2.827           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr10   | 1      | 19.277  | 20           | 2.004           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr11   | 1      | 21.555  | 20           | 2.188           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr12   | 1      | 27.485  | 5            | 4.656           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr13   | 1      | 28.180  | 5            | 5.128           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr14   | 1      | 34.646  | 5            | 4.134           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr15   | 1      | 30.651  | 5            | 4.142           | 500          |                 |
| Festuca   | Phlegethon australis      | PhleAr16   | 1      | 28.647  | 5            | 3.638           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr1    | 1      | 63.130  | 20           | 8.141           | 500          | 61.224          |
| Festuca   | Deichmantha cephalotes    | DeicAr2    | 1      | 60.713  | 5            | 3.124           | 500          | 1.653           |
| Festuca   | Deichmantha cephalotes    | DeicAr3    | 1      | 61.260  | 5            | 4.138           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr4    | 1      | 71.738  | 5            | 5.148           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr5    | 1      | 68.668  | 5            | 6.716           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr6    | 1      | 51.443  | 5            | 2.138           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr7    | 1      | 56.627  | 5            | 8.880           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr8    | 2      | 1.680   | 5            | 1.780           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr9    | 1      | 60.655  | 5            | 4.132           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr10   | 1      | 2.623   | 5            | 2.187           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr11   | 3      | 70.796  | 5            | 9.722           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr12   | 1      | 13.166  | 5            | 7.024           | 500          |                 |
| Festuca   | Deichmantha cephalotes    | DeicAr13   | 1      | 18.840  | 5            | 4.980           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr1   | 1      | 134.045 | 10           | 6.134           | 500          | 62.249          |
| Festuca   | Echinochloa crus-galli    | EchCrAr2   | 2      | 72.143  | 10           | 5.531           | 500          | 4.403           |
| Festuca   | Echinochloa crus-galli    | EchCrAr3   | 1      | 60.022  | 10           | 6.134           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr4   | 1      | 80.423  | 5            | 3.647           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr5   | 1      | 10.251  | 5            | 2.138           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr6   | 2      | 81.827  | 5            | 5.148           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr7   | 1      | 54.056  | 5            | 6.134           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr8   | 2      | 42.863  | 5            | 2.138           | 700          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr9   | 1      | 73.855  | 5            | 3.128           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr10  | 1      | 54.484  | 5            | 4.408           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr11  | 1      | 36.103  | 10           | 5.176           | 650          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr12  | 1      | 43.830  | 10           | 6.134           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr13  | 1      | 48.077  | 10           | 5.147           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr14  | 1      | 46.266  | 10           | 4.134           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr15  | 1      | 3.193   | 10           | 3.193           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr16  | 1      | 34.117  | 5            | 2.633           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr17  | 1      | 2.185   | 5            | 4.137           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr18  | 1      | 60.265  | 10           | 4.709           | 500          |                 |
| Festuca   | Echinochloa crus-galli    | EchCrAr19  | 1      | 41.600  | 5            | 6.075           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr1  | 1      | 37.721  | 5            | 2.838           | 500          | 66.854          |
| Festuca   | Narditis stricta          | NardStAr2  | 1      | 1.613   | 5            | 4.130           | 500          | 5.220           |
| Festuca   | Narditis stricta          | NardStAr3  | 1      | 41.871  | 5            | 3.134           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr4  | 1      | 40.878  | 5            | 4.140           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr5  | 1      | 71.738  | 10           | 6.138           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr6  | 1      | 40.246  | 10           | 3.647           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr7  | 2      | 72.137  | 10           | 6.146           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr8  | 3      | 74.828  | 10           | 7.125           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr9  | 1      | 51.642  | 10           | 3.675           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr10 | 2      | 42.892  | 10           | 4.138           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr11 | 3      | 68.835  | 10           | 6.499           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr12 | 4      | 73.655  | 10           | 5.133           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr13 | 1      | 67.978  | 10           | 4.908           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr14 | 2      | 65.880  | 10           | 6.133           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr15 | 1      | 68.282  | 10           | 6.032           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr16 | 1      | 60.174  | 10           | 5.134           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr17 | 1      | 70.123  | 10           | 6.134           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr18 | 1      | 41.831  | 10           | 4.031           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr19 | 1      | 71.422  | 10           | 7.125           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr20 | 1      | 86.236  | 10           | 7.165           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr21 | 4      | 81.748  | 10           | 7.166           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr22 | 1      | 81.404  | 10           | 4.138           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr23 | 1      | 2.402   | 10           | 6.132           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr24 | 1      | 68.054  | 10           | 4.138           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr25 | 1      | 51.595  | 5            | 2.164           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr26 | 1      | 51.261  | 5            | 4.428           | 500          |                 |
| Festuca   | Narditis stricta          | NardStAr27 | 1      | 101.862 | 10           | 11.125          | 500          |                 |
| Festuca   | Avena sativa              | AvenSaAr1  | 1      | 102.842 | 5            | 6.280           | 700          |                 |
| Festuca   | Avena sativa              | AvenSaAr2  | 1      | 138.180 | 5            | 6.542           | 650          |                 |
| Festuca   | Avena sativa              | AvenSaAr3  | 1      | 138.180 | 5            | 8.226           | 700          |                 |
| Festuca   | Avena sativa              | AvenSaAr4  | 1      | 91.840  | 5            | 5.090           | 500          |                 |
| Festuca   | Avena sativa              | AvenSaAr5  | 1      | 122.471 | 5            | 6.175           | 500          |                 |
| Festuca   | Avena sativa              | AvenSaAr6  | 1      | 112.831 | 10           | 11.112          | 700          | 146.312         |
| Festuca   | Avena sativa              | AvenSaAr7  | 1      | 112.383 | 10           | 10.127          | 700          | 10.377          |
| Festuca   | Avena sativa              | AvenSaAr8  | 1      | 90.840  | 10           | 10.870          | 700          |                 |
| Festuca   | Avena sativa              | AvenSaAr9  | 1      | 101.021 | 10           | 10.174          | 700          |                 |
| Festuca   | Avena sativa              | AvenSaAr10 | 1      | 174.618 | 5            | 13.612          | 500          |                 |
| Festuca   | Avena sativa              | AvenSaAr11 | 1      | 151.341 | 5            | 11.111          | 500          |                 |
| Festuca   | Avena sativa              | AvenSaAr12 | 1      | 94.144  | 5            | 7.111           | 700          |                 |
| Festuca   | Briza media               | BrizMeAr1  | 1      | 81.738  | 20           | 6.134           | 200          | 95.023          |
| Festuca   | Briza media               | BrizMeAr2  | 2      | 88.658  | 2            | 4.034           | 200          | 6.101           |
| Festuca   | Briza media               | BrizMeAr3  | 1      | 111.890 | 2            | 6.134           | 200          |                 |
| Festuca   | Briza media               | BrizMeAr4  | 1      | 163.479 | 2            | 9.208           | 500          |                 |
| Festuca   | Briza media               | BrizMeAr5  | 1      | 81.979  | 2            | 5.140           | 500          |                 |
| Festuca   | Briza media               | BrizMeAr6  | 1      | 79.046  | 2            | 5.034           | 500          |                 |
| Festuca   | Briza media               | BrizMeAr7  | 1      | 70.143  | 2            | 6.134           | 500          |                 |
| Festuca   | Briza media               | BrizMeAr8  | 1      | 70.246  | 2            | 5.123           | 500          |                 |
| Festuca   | Briza media               | BrizMeAr9  | 1      | 81.837  | 2            | 5.175           | 500          |                 |
| Festuca   | Briza media               | BrizMeAr10 | 1      | 71.248  | 2            | 4.480           | 500          |                 |
| Festuca   | Brachypodium pinnatifidum | BrachPiAr1 | 1      | 14.711  | 10           | 1.123           | 500          | 20.830          |
| Festuca   | Brachypodium pinnatifidum | BrachPiAr2 | 1      | 10.154  | 10           | 1.904           | 500          | 1.865           |
| Festuca   | Brachypodium pinnatifidum | BrachPiAr3 | 1      | 14.838  | 10           | 1.023           | 500          |                 |
| Festuca   | Brachypodium pinnatifidum | BrachPiAr4 | 1      | 17.411  | 10           | 1.752           | 700          |                 |
| Festuca   | Brachypodium pinnatifidum | BrachPiAr5 | 1      | 18.438  | 10           | 1.904           | 500          |                 |
| Festuca   | Brachypodium pinnatifidum | BrachPiAr6 | 1      | 24.448  | 10           | 1.766           | 650          |                 |
| Festuca   | Brachypodium pinnatifidum | BrachPiAr7 | 1      | 20.955  | 10           | 1.142           | 500          |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr1   | 1      | 68.201  | 4,333        | 40              | 30           | 95.318          |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr2   | 1      | 128.56  | 7,841        | 30              | 30           | 5.207           |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr3   | 1      | 96.177  | 2,432        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr4   | 1      | 84.617  | 4,809        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr5   | 1      | 114.012 | 7,41         | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr6   | 1      | 82.437  | 3,968        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr7   | 1      | 91.487  | 4,009        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr8   | 1      | 81.486  | 4,181        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr9   | 1      | 76.8    | 4,375        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr10  | 1      | 81.486  | 4,009        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr11  | 1      | 101.385 | 6,008        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr12  | 1      | 203.762 | 4,128        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr13  | 1      | 40.997  | 2,185        | 40              | 30           | 80.782          |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr14  | 1      | 41.981  | 3,587        | 40              | 30           | 5.567           |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr15  | 1      | 100.219 | 5,023        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr16  | 1      | 81.207  | 5,368        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr17  | 1      | 70.813  | 4,758        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr18  | 1      | 71.613  | 5,127        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr19  | 1      | 77.809  | 4,656        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr20  | 1      | 72.381  | 5,275        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr21  | 1      | 110.121 | 6,409        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr22  | 1      | 112.174 | 6,897        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr23  | 1      | 40.758  | 3,095        | 40              | 30           |                 |
| Agropyron | Hordeopyrum pentstemonis  | HorpeAr24  | 1      | 112.265 | 4,541        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr1  | 1      | 134.46  | 1,227        | 40              | 30           | 95.229          |
| Agropyron | Agropyrum holcoides       | AgroHoAr2  | 2      | 80.132  | 1,651        | 40              | 30           | 5.408           |
| Agropyron | Agropyrum holcoides       | AgroHoAr3  | 1      | 108.18  | 2,384        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr4  | 1      | 87.72   | 1,622        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr5  | 1      | 121.008 | 2,761        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr6  | 1      | 112.172 | 3,240        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr7  | 1      | 82.138  | 1,009        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr8  | 1      | 111.626 | 4,006        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr9  | 1      | 112.127 | 4,852        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr10 | 1      | 111.026 | 4,008        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr11 | 1      | 100.827 | 3,865        | 40              | 30           |                 |
| Agropyron | Agropyrum holcoides       | AgroHoAr12 | 1      |         |              |                 |              |                 |

**Supplementary Figure S1:** CenH3 immunolabeling of the grass species



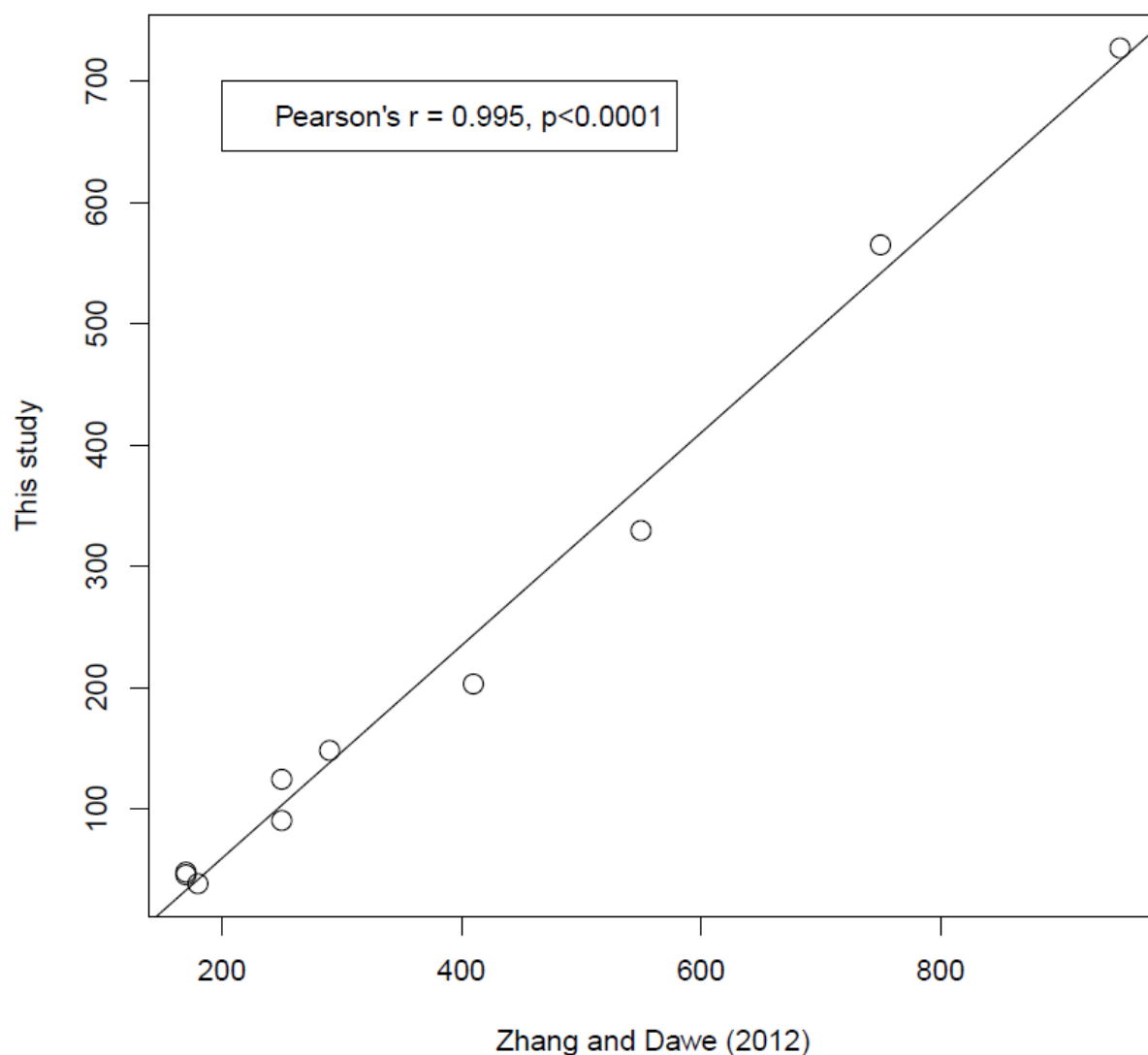
Interphase nuclei of grass (Poaceae) species with DAPI-stained DNA (blue) and immuno-labeled CENH3 (green). Species are ordered according to their genome size from the smallest to the largest. A=*Brachypodium sylvaticum*, B=*Phragmites australis*, C=*Echinochloa crus-galli*, D=*Nardus stricta*, E=*Melica uniflora*, F=*Deschampsia cespitosa*, G=*Avenella flexuosa*, H=*Briza media*.

**Supplementary Figure S2:** CenH3 immunolabeling of Agavoideae species



Interphase nuclei of Agavoideae (Asparagaceae) species with DAPI-stained DNA (blue) and immunolabeled CENH3 (green). Species are ordered according to their genome size from the smallest to the largest. A=*Yucca elata*, B=*Yucca constricta*, C=*Yucca carnerosana*, D=*Hesperaloe funifera*, E=*Hesperaloe parviflora*, F=*Agave tequilana*, G=*Hesperoyucca whipplei*, H=*Hesperoyucca peninsularis*, I=*Agave toumeyana*.

**Supplementary Figure S3:** Comparison of our and Zhang and Dawe's (2012) measurements of the total centromere size



The total centromere size values from Zhang and Dawe (2012) were read from their Figure 2. The values from our study were measured as described in the Methods section and are listed in Supplementary Table S1. The species included are: *Avena sativa*, *Hordeum vulgare*, *Pennisetum glaucum*, *Oryza sativa*, *Secale cereale*, *Setaria italica*, *Sorghum bicolor*, *Triticum aestivum*, *Zea luxurians*, *Zea mays*.

Reference: Zhang H, Dawe RK. 2012. Total centromere size and genome size are strongly correlated in ten grass species. *Chromosome Res* 20: 403-412. doi:10.1007/s10577-012-9284-1

## Supplementary Text S1. Supplementary Methods

### *Cytological preparation (immunostaining)*

Root tips from grass and agavoides species were fixed for 25 min in ice-cold 4% (w/v) formaldehyde diluted in 1x PBS buffer (pH = 7.4), washed (3x 15 min in 1x PBS), and digested in an enzyme mix containing 0.085% (w/v) cellulase R-10 (Duchefa Biochemie), cellulysin cellulase (Merck Millipore) and 0.12% (w/v) pectolyase Y23 (Duchefa Biochemie), cytohelicase (Sigma-Aldrich) in 1 x PBS for 60 to 90 min at 37°C. The nuclear spreads were performed in 1x PBS and slides were immersed into liquid nitrogen. Slides were incubated with 100 µl 4% BSA in 1x PBS for one hour at room temperature and then with 80 µl primary antibodies [grass species with anti-OsCENH3 (Nagaki *et al.*, 2004) diluted in 1x PBS, 1:50 to 1:100 and Agavoideae species with anti-Agavoideae-CenH3 diluted in 1xPBS, 1:250 to 1:500] overnight at 4°C. After washing (3x 5 min) slides were treated with 80 µl secondary antibody (Anti-Rabbit Alexa 488, diluted in 1x PBS, 1:500) for one hour at 37°C. After washing (3x 5 min) and dehydration using a graded ethanol series, nuclei were mounted with 8 µl DAPI (1.5 µg/ml) in Vectashield.

### *Generation of anti-Agavoideae-CenH3 Antibody*

The peptide MARVKHKPQPQPRRRLVLNEA was used for the production of polyclonal antibodies in rabbits. We selected the peptide and the peptide synthesis, immunization of rabbits, and peptide affinity purification of antisera was performed by LifeTein company (USA, [www.lifetein.com](http://www.lifetein.com)).

### *Flow cytometry*

Genome size of Agavoideae species was measured with flow cytometry using standard protocols consistently applied in our laboratory over the last decade (see Šmarda *et al.* 2008 and Šmarda *et al.*, 2019 for details).

## References

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## Supplementary Text S2. Dated phylogenetic tree in Newick format

(Giardia\_intestinalis:2045.118249,((Cyanidioschyzon\_merolae:1602.266792,((((Schizosaccharomyces\_pombe:590,Saccharomyces\_cerevisiae:590,(Magnaporthe\_oryzae:245,Neurospora\_crassa:245)10:345,Fusarium\_graminearum:590,((Candida\_tropicalis:39,Candida\_albicans:39)12:83,Candida\_auris:122)11:468)9:133,Cryptococcus\_neoformans:723)8:383.7575556,(((Xenopus\_laevis:354.5891499,(((Equus\_caballus:7.72,Equus\_asinus:7.72)19:70.28,Muntiacus\_muntjac:78)18:14.60238917,(((Microcebus\_murinus:55,Daubentonia\_madagascariensis:55)22:18.83689435,((Callithrix\_jacchus:19.7,(Cebus\_apella:16.1,Saimiri\_sciureus:16.1)27:3.6)24:23.45129635,Homo\_sapiens:43.15129635)23:30.685598)21:16.16310565,Mus\_musculus:90)20:2.60238917)17:222.430468,Gallus\_gallus:315.0328571)16:39.55629277)15:83.0646001,(Latescalcifer:110,Gasterosteus\_aculeatus:110)29:327.65375)14:361.2607867,((((Drosophila\_melanogaster:50,Drosophila\_virilis:50)34:222,Aedes\_albopictus:272)33:38,Tribolium\_castaneum:310)32:433,(Meloidogyne\_incognita:203.7,Caenorhabditis\_elegans:203.7)35:539.3)31:10,Crassostrea\_gigas:753)30:45.91453672)13:307.8430188)7:310.3513524,Dictyostelium\_discoideum:1417.108908)6:0,((((Hesperoyucca\_pensularis:1,Hesperoyucca\_whipplei:1)41:10.9,(Hesperaloe\_funifera:2.02,Hesperaloe\_parviflora:2.02)42:9.88)40:3.79544,(Yucca\_carnerosana:6.4,(Yucca\_elata:5.3,Yucca\_constricta:5.3)45:1.1)44:2.7,(Agave\_toumeyana:1.53,Agave\_tequilana:1.53)46:7.57)43:6.59544)39:42.90456,(((Allium\_fistulosum:2.66,Allium\_cepa:2.66)49:6.363,Allium\_sativum:9.023)48:7.074,Allium\_tuberosum:16.097)47:42.503)38:55.4,((((Oryza\_granulata:13.2,Oryza\_brachyantha:13.2)54:2.09875,((Oryza\_punctata:5.7,Oryza\_sativa:5.7)56:0.4528,(Oryza\_officinalis:1.26466667,Oryza\_rhizomatis:1.26466667)57:4.88813333)55:9.14595)53:34.30125,((((Secale\_cereale:10.9,Hordeum\_vulgare:10.9)63:0,(Triticum\_aestivum:3.55556667,Aegilops\_speltoides:3.55556667)64:7.34443333)62:13.20373857,((Briza\_media:13.6,Avena\_sativa:13.6)66:8.98926714,(Avenella\_flexuosa:17.46867,((Festuca\_maurei:12.4,Lolium\_multiflorum:12.4)69:3,Deschampsia\_cespitosa:15.4)68:2.06867)67:5.12059714)65:1.51447143)61:4.98136143,Melica\_uniflora:29.0851)60:1.41029,Nardus\_stricta:30.49539)59:2.08636444,(Brachypodium\_sylvaticum:9.9,Brachypodium\_distachyon:9.9)70:22.68175444)58:17.01824556)52:2.913225217e-13,(((Echinochloa\_crusgalli:18.59326,((Setaria\_italica:6.3,Pennisetum\_glaucum:6.3)75:6.5,Panicum\_virgatum:12.8)74:5.79326)73:4.80674,((Sorghum\_bicolor:10.4,(Saccharum\_officinarum:2.75,Saccharum\_spontaneum:2.75)78:7.65)77:1.79144143,Coix\_lacryma-jobi:12.19144143,(Zea\_mays:0.14,Zea\_luxurians:0.14)79:12.05144143)76:11.20855857)72:13.46000286,Phragmites\_australis:36.86000286)71:12.73999714)51:45.37617111,((((Luzula\_luzuloides:5,Luzula\_nivea:5)83:32,Luzula\_elegans:37)82:37.85935889,(((Rhynchospora\_pubera:17,Rhynchospora\_ciliata:17)86:3,Rhynchospora\_tenuis:20)85:10,Rhynchospora\_cephalotes:30)84:44.85935889)81:8.14064111,Prionium\_serratum:83)80:11.97617111)50:19.02382889)37:45.78069088,(Nelumbo\_nucifera:121.2857063,((((Gossypium\_hirsutum:85.94042417,(((Brassica\_rapa:2.02,(Brassica\_juncea:1.14,Brassica\_napus:1.14)95:0.88)94:7.29915167,Raphanus\_sativus:9.31915167)93:16.28084833,Arabidopsis\_thaliana:25.6)92:60.34042417)91:9.06565369,Citrus\_clementina:95.00607786)90:7.506932574,(((Glycine\_max:23.9983,(Vigna\_unguiculata:10.4,Phaseolus\_vulgaris:10.4)100:13.5983)99:29.21281111,((((Vicia\_faba:8.0205,Vicia\_sativa:8.0205)104:5.50155,Vicia\_annonica:13.52205)103:13.67795,Lens\_culinaris:27.2)102:0.0199999995,((Pisum\_fulvum:1.81,Pisum\_sativum:1.81)106:15.42333333,((Lathyrus\_sylvestris:9.8381,Lathyrus\_sativus:9.8381)108:7.3119,Lathyrus\_vernus:17.15)107:0.08333333167)105:9.986666668)101:25.99111111)98:5.81416111,Lotus\_japonicus:59.02527222)97:43.48773821)89:13.18883801,(((Beta\_vulgaris:1.416549,Beta\_maritima:1.416549)111:0.167511,Beta\_patula:1.58406)110:109.9224598,(((Daucus\_carota:13.4,Daucus\_glochidiatius:13.4)114:78.81183667,Helianthus\_annuus:92.21183667)113:11.78816333,(((Nicotiana\_tomentosiformis:10.69743,(Nicotiana\_tabacum:3.871105,Nicotiana\_sylvestris:3.871105)118:6.826325)117:19.10257,Solanum\_tuberosum:29.8)116:36.849802,(Cuscuta\_japonica:20.5,((((Cuscuta\_pentagona:1,Cuscuta\_campestris:1)250:2.4,Cuscuta\_californica:3.4)249:0.8,Cuscuta\_gronovii:4.2)123:1.1,Cuscuta\_americana:5.3)122:3.1,Cuscuta\_indecora:8.4)121:8.6,Cuscuta\_europea:17)120:3.5)119:46.149802)115:37.350198)112:7.50651981)109:4.195328639)88:5.583857844)87:38.49498459)36:1257.328217)5:184.7068429,Trichomonas\_vaginalis:1601.815751)4:0.4510408459)3:108.8619415,(Tetrahymena\_thermophila:1290,Phytophthora\_sojae:1290,(Plasmodium\_falciparum:817.175,Toxoplasma\_gondii:817.175)252:472.825)251:421.1287332)2:333.9895158)1;