Perpetual Flowering in Strawberry Species

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Abstract. The genetic control of flowering habit in many species of *Fragaria* has not been well studied. Identification of flowering traits and patterns for these taxa could be used in the quest for perpetual flowering (PF) genes and for the octoploids, broaden the genepool of available PF parents for breeding programs. As such, clones from the *Fragaria* germplasm collection housed at the USDA-ARS National Clonal Germplasm Repository in Corvallis, OR, were evaluated to describe flowering habits in various taxa and identify PF clones. Flower presence was recorded monthly for 962 clones of 36 taxa from the first of May through October in 2015 and 2016 to determine flowering habit and pairwise comparisons between taxa were examined using Pearson's Chi-squared test. Taxa with the largest percent of PF accessions were *F. vesca* subsp. *vesca* f. *semperflorens*, *F. vesca* subsp. *vesca* f. *alba*, *F. vesca* subsp. *americana*, and *F. virginiana* subsp. *glauca*. These taxa had similar flowering habits to each other but were significantly different ($\alpha = 0.05$) from most other taxa in which the seasonal flowering (SF) trait was predominant. Fifteen clones that demonstrated the PF phenotype in both 2015 and 2016 were identified. Differing genetic controls have been observed for flowering habit in *F. ×ananassa* and *F. vesca*. Additional studies are needed to determine genetic control of flowering in other *Fragaria* taxa.

The cultivated octoploid garden strawberries, *Fragaria ×ananassa*, are classified based on their flowering habit into those that flower and fruit mostly once in the Spring and those that continue to flower and fruit as long as temperatures are moderate (below 30/26 °C day/night) (Darrow, 1966; Hancock, 1999). Multiple terms have been used interchangeably to describe strawberry flowering habit. “Short day,” “once flowering,” “seasonal flowering,” “single cropping plants,” or “June-bearing” has been used to describe plants which bloom in the spring and produce one fruit crop per summer. The terms “everbearing,” “remontant,” “day-neutral,” “perpetual flower-

ing,” and “long day plants” have all been used to describe plants which flower multiple times and produce multiple crops over the course of the summer. We chose the terms “seasonal flowering” (SF) and “perpetual flowering” (PF) to describe flowering habit in this study (Gaston et al., 2013).

During most of the 1900s, the cultivars produced were SF, blooming in the spring and producing one fruit crop per summer. PF forms of the diploid alpine strawberry, *F. vesca* subsp. *vesca* f. *semperflorens*, were common in Europe when Linnaeus named the genus (Duchesne, 1766). *F. ×ananassa* with the PF trait were recorded since the mid 1860s. ‘Gloede’s seedling’ was the first widely known PF garden strawberry (Richardson, 1914). Others, such as ‘Laxton’s Perpetual’, ‘Mastadon’, and ‘Rockhill’, were early parental types for this trait (Clark, 1937; Powers, 1954; Richardson, 1914). These cultivars that extended the fruit production season and increased productivity were termed “everbearing.” The discovery by Bringhurst in 1955, of what became known as the “day-neutral” *F. virginiana* subsp. *glauca* clone from the Wasatch Mountains in Utah, was a pivotal event causing changes in strawberry production practices in California and the world (Bringhurst et al., 1989, 1990; Faedi et al., 2002; Salinas et al., 2017; Simpson, 1993; Zurawicz and Masny, 2002). This clone was the key in producing a family

of PF releases from the University of California strawberry breeding program.

The genetics behind PF are of considerable economic interest to growers, the food industry, and global consumers, who, with increased global production, can now enjoy strawberries every day of the year. Studies have identified genes or loci underlying the control of flowering habit in the diploid alpine and in the common garden strawberry (Gaston et al., 2013; Iwata et al., 2012; Koskela et al., 2012; Perrotte et al., 2016a, 2016b; Salinas et al., 2017). In the alpine strawberry, flowering habit is governed by the floral repressor *FvTFL1* located on chromosome 6 (Iwata et al., 2012; Koskela et al., 2012). *FvTFL1* suppresses the flowering inducing gene, *Flowering Locus T*, *FvFTL*, which is also located on chromosome 6 (Iwata et al., 2012; Koskela et al., 2012). A 2 bp deletion in *FvTFL1* prevents it from suppressing *FvFTL*, resulting in PF (Iwata et al., 2012; Koskela et al., 2012). In the common garden strawberry, PF can be induced by silencing *FaTFL1*, an *FvTFL1* homolog; however, *FaTFL1* is not responsible for the PF phenotype conferred by the Wasatch source (Nakano et al., 2015; Perrotte et al., 2016a). The Wasatch source of PF is conferred by the *FaPFRU* locus on chromosome 4B (Gaston et al., 2013; Perrotte et al., 2016a). While the gene controlling PF is not known, a homolog of *FvFT2* was proposed as the most likely candidate (Gaston et al., 2013; Perrotte et al., 2016a). Interestingly, *FaPFRU* inversely controls both flowering and runnering (Gaston et al., 2013; Perrotte et al., 2016a). The dominant allele of *FaPFRU* induces more inflorescences than stolons, and in homozygous recessive plants, more stolons than inflorescences are produced given the study conditions (Gaston et al., 2013). Despite the body of work that has been done in *F. vesca* and the Wasatch source of PF in *F. ×ananassa*, very little is known about the genetics underlying flowering habit in other *F. ×ananassa* PF sources or *Fragaria* taxa.

Seasonal cues, such as temperature, light conditions, and daylength, can have large effects on the occurrence of PF (Andrés and Coupland, 2012; Salinas et al., 2017). Under different temperature conditions, the flowering habit of strawberries has been observed to be under either qualitative or quantitative genetic control (Sønsteby and Heide, 2007). Moreover, under long-day conditions (>14 h daylight), SF individuals behave as PF plants at low temperatures (Darrow, 1936; Darrow and Waldo, 1934). The PF trait can also occur where latent buds develop through the removal of inflorescences during the growing season (Sugiyama et al., 2004).

Within the genus *Fragaria*, 22 species, multiple species hybrids, and numerous subspecies have been globally described (Liston et al., 2014). Identification of flowering traits for individuals of these taxa could broaden the genepool of available PF parents for breeding programs. A recent study examined recurrent bloom of American octoploid

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strawberry species (Hummer et al., 2016). The present study is an expansion of that work. The objectives of this project were to screen diverse strawberry genetic resources for flowering phenotype and to identify particular PF clones. Innovative breeders are using secondary and tertiary gene pools in their crosses as well as introgressing traits from new wild collections within the primary gene pool to seek improvements and berries with novel traits (Hancock et al., 2010). This study could expand taxa or particular individuals for consideration as parents where PF is desirable.

Materials and Methods

Germplasm. For this phenotyping study, 962 clones from 38 strawberry taxa and hybrids were observed at the USDA-ARS National Clonal Germplasm Repository (NCGR) in Corvallis, OR (Table 1). These taxa represent diploids, tetraploids, octoploids, and decaploids distributed across the two taxonomic clades identified by Liston et al. (2014) and Tennesen et al. (2014). Of the 38 taxa, 10 had low representation with four or fewer samples being available at the NCGR (Table 1). Precise temperature data within the screenhouses where the straw-

berries were growing was unavailable for this study. Temperatures from the local Corvallis, OR, airport for 2015 and 2016 were used to estimate regional cumulative growing degree days and mean daily temperatures (Fig. 1, NOAA, 2017). Growing Degree Day units were computed as the difference between the daily average temperature and the base temperature (Daily Ave. Temp. – Base Temp.). One unit is accumulated for each degree Fahrenheit when the average temperature is above the base temperature. Negative numbers were discarded. This was done for each day of the months from January through June and summed for each year.

Phenotyping. Flower presence was recorded in 2015 and 2016, on the first day of the month from May through October. Inflorescences and complete trusses were removed after scoring. Plants flowering only before August first were considered SF, and those flowering on and after August first were considered PF. The cut-off date chosen represented the first date of flowering evaluation about six weeks after the longest day of the year, 21 June.

Statistical analysis. The 38 taxa, including hybrids, were separated into groups consisting of taxa with more than four representatives (28 taxa) and taxa with less than four repre-

sentatives (10 taxa; Table 1). For the 28 taxa with greater than four individuals, two-by-two contingency tables were created to conduct comparisons of the flowering data where the two categories were “species” and “flowering habit,” either PF or SF. Pearson’s Chi-squared test with the ‘N-1’ correction (Campbell, 2007) was used to determine if the flowering habits of species differed. The Benjamini–Hochberg method was used to control for experiment-wide false discovery rate during pairwise comparisons (Benjamini and Hochberg, 1995). Calculations were performed in R version 3.2.5 (R Core Team, 2016) using a custom R script (Supplemental file 1).

Results

Many of the taxa had individuals that were PF; however, flowering habit for the taxon as a whole differed (Table 1). The most common flowering habit for the genus *Fragaria* appeared to be SF, with 24.1% and 35.3% of the accessions exhibiting PF phenotypes for 2015 and 2016, respectively. *Fragaria bucharica*, *F. moschata*, *F. virginiana* subsp. *glauca*, and some members of *F. vesca* were similar and tended to have different flowering habits than other taxa

Table 1. Perpetual flowering (PF) in 38 *Fragaria* taxa and hybrids in 2015 and 2016 in screenhouses at the USDA National Clonal Germplasm Repository in Corvallis, OR.

Taxon	No. Genotypes	No. PF 2015	% PF 2015	No. PF 2016	% PF 2016
Taxa where $N > 4$					
<i>F. nilgerrensis</i>	9	0	0	0	0
<i>F. virginiana</i> subsp. <i>grayana</i>	50	1	2	4	8
<i>F. chiloensis</i> subsp. <i>lucida</i>	20	1	5	2	10
<i>F. xananassa</i> subsp. <i>cuneifolia</i>	18	1	5.6	1	5.6
<i>F. xbringhurstii</i>	17	1	5.9	1	5.9
<i>F. chiloensis</i> subsp. <i>pacifica</i>	39	3	7.7	4	10.3
<i>F. iinumae</i>	23	2	8.7	2	8.7
<i>F. nipponica</i>	11	3	27.3	1	9.1
<i>F. chiloensis</i> subsp. <i>chiloensis</i> f <i>chiloensis</i>	17	2	11.8	5	29.4
<i>F. virginiana</i> subsp. <i>virginiana</i>	273	56	20.5	67	24.5
<i>F. viridis</i>	8	0	0	4	50
<i>F. corymbosa</i>	4	1	25	1	25
<i>F. orientalis</i>	4	1	25	1	25
<i>F. pentaphylla</i>	4	1	25	1	25
<i>F. virginiana</i> var. <i>platypetala</i>	52	12	23.1	17	32.7
<i>F. hybrid</i> (<i>F. iinumae</i> × <i>F. nipponica</i>)	16	4	25	6	37.5
<i>F. chiloensis</i> subsp. <i>chiloensis</i> f <i>patagonica</i>	169	25	14.8	81	47.9
<i>F. cascadiensis</i>	36	17	47.2	11	30.6
<i>F. vesca</i> subsp. <i>californica</i>	7	2	28.6	5	71.4
<i>F. xananassa</i>	25	5	20	17	68
<i>F. vesca</i> subsp. <i>bracteata</i> f <i>bracteata</i>	53	19	35.8	28	52.8
<i>F. vesca</i> subsp. <i>vesca</i>	11	4	36.4	9	81.8
<i>F. moschata</i>	5	3	60	3	60
<i>F. bucharica</i>	4	3	75	3	75
<i>F. virginiana</i> subsp. <i>glauca</i>	37	31	83.8	29	78.4
<i>F. vesca</i> subsp. <i>americana</i>	18	13	72.2	17	94.4
<i>F. vesca</i> subsp. <i>vesca</i> f <i>semperflorens</i>	8	8	100	8	100
<i>F. vesca</i> subsp. <i>vesca</i> f <i>alba</i>	5	5	100	5	100
Taxa where $N < 4$					
<i>F. chiloensis</i> subsp. <i>sandwicensis</i>	2	0	0	0	0
<i>F. nubicola</i>	2	0	0	0	0
<i>F. moupinensis</i>	2	0	0	1	50
<i>F. iturupensis</i>	3	2	66.7	0	0
<i>F. chinensis</i>	3	1	33.3	2	66.7
<i>F. xbfifera</i>	2	1	50	1	50
<i>F. daltoniana</i>	1	1	100	0	0
<i>F. gracilis</i>	1	0	0	1	100
<i>F. mandshurica</i>	2	2	100	1	50
<i>F. tibetica</i>	1	1	100	1	100

($\alpha = 0.1$) with a greater proportion of PF individuals being present (Fig. 2; Table 1). The western diploids, *F. vesca* subsp. *californica*

Fig. 1. Cumulative growing degree day units (A), base 50 °F (10 °C), and the mean daily temperature (B) for the first 6 months of 2015 and 2016 in Corvallis, OR (NOAA, 2017).

and *F. vesca* subsp. *bracteata*, were the exceptions compared with other *F. vesca*, with most individuals being SF (Fig. 2; Table 1).

The Asian species *F. iinumae*, *F. nilgerrensis*, *F. viridis*, *F. nubicola*, *F. daltoniana*, *F. chinensis*, *F. mandshurica*, *F. corymbosa*, *F. moupinensis*, *F. orientalis*, and *F. pentaphylla* tended to be SF (Table 1). No conclusions could be drawn about *F. tibetica* and *F. gracilis* because only one clone was available for observation for each taxon.

Clones of most of the American octoploid subspecies of *F. virginiana* and *F. chiloensis* were SF with the exception of *F. virginiana* subsp. *glauca* (Fig. 2; Table 1). The South American *F. chiloensis* subsp. *chiloensis* f. *patagonica* had the highest percentage of PF clones of any *F. chiloensis* subspecies examined in both 2015 and 2016. The higher early spring temperatures may have encouraged more PF events in the *F. chiloensis* clones in 2016 compared with the temperatures of 2015. The two clones of the Hawaiian *F. chiloensis* subsp. *sandwicensis* were SF and did not rebloom in either year.

As might be expected, hybrid species tended to have mixed responses for flowering habit. *Fragaria ×ananassa* subsp. *cuneifolia*, a naturally occurring hybrid of the North

American beach strawberry and the Virginia strawberry, was SF (Table 1). A set of native hybrid *F. nipponica* × *F. iinumae* diploid genotypes from Hokkaido was predominantly SF. The response of *F. ×bifera*, determined to be the natural hybrid of *F. vesca* × *F. viridis* (Staudt et al., 2003), was 50%; one clone was PF and the other SF.

Fifteen clones from three species, *F. chiloensis*, *F. vesca*, and *F. virginiana*, bloomed seasonally and during August, September, and October of both 2015 and 2016. These clones had the strongest PF tendency out of the 962 clones observed (Table 2). While this was not unexpected for *F. vesca* subsp. *vesca*, finding strong PF tendency in *F. chiloensis* subsp. *patagonica* from Chile, *F. virginiana* subsp. *glauca* from Alaska, MT, and Idaho, *F. virginiana* subsp. *virginiana* from Quebec and Minnesota, and *F. virginiana* subsp. *platypetala* from Oregon, was notable. The Minnesota clones were also reported as day neutral by Hancock et al. (2002).

Discussion

Clark (1937) called for extensive experimentation to obtain satisfactory explanation of the genetic behavior of PF cultivars and the complex polyploid nature of strawberry species. This question still resonates. The genes underlying flowering habit have only recently begun to be studied in *F. vesca* and *F. ×ananassa* (Iwata et al., 2012; Koskela et al., 2012; Nakano et al., 2015; Perrotte et al., 2016a). Each of the genes identified has homology to flowering genes in *Arabidopsis*, however, the PF phenotypes in *F. vesca* and *F. ×ananassa* appear to be mediated through different pathways (Iwata et al., 2012; Koskela et al., 2012; Nakano et al., 2015; Perrotte et al., 2016a). Moreover, the genes mediating the PF phenotype in *Fragaria* species other than *F. vesca* and *F. ×ananassa* are yet to be studied in great detail. As such, 962 clones of individuals from 38 *Fragaria* taxa and hybrids were observed to better characterize flowering habit and identify individuals for future study.

The European vesca types, *F. vesca* subsp. *vesca* f. *semperflorens* and *F. vesca* f. *alba*, and the eastern American *F. vesca* subsp. *americana*, tended to be PF; whereas a second group consisting of the western American *F. vesca* subsp. *bracteata* and *F. vesca* subsp. *californica* was predominantly SF. This is consistent with Ahmadi et al. (1990). Both Tennessen et al. (2014) and Njuguna et al. (2013) found similar genetic differentiation within *F. vesca*, whereas the western North American vesca subspecies were distinct from the eastern North American *F. vesca* subsp. *americana*, which grouped with the European *F. vesca*. The PF habit of *F. vesca* subsp. *vesca* f. *semperflorens* and *F. vesca* f. *alba* was expected. These taxa have been noted for their PF habit since they were described in the 1700s (Duchesne, 1766). Both forms were European natives that were introduced in many parts of the world and early European

Fig. 2. Heat map depicting the pairwise comparison of flowering habit for 28 *Fragaria* taxa plotted for 2015 (bottom left) and 2016 (upper right) using Pearson's Chi-squared test with an 'N-1' correction (Campbell, 2007). Experiment-wide error was controlled using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). The taxa presented in the figure were arranged based on clades as defined by Liston et al. (2014).

Table 2. The 15 strongest perpetual flowering (PF) *Fragaria* genotypes that bloomed in August, September, and October in both 2015 and 2016 in screenhouses at the USDA-ARS National Clonal Germplasm Repository in Corvallis, OR. Sex of the accessions is listed as hermaphroditic (H) or female (F).

Accession	CFRA ² no.	Taxon	Name	Sex	Location collected
PI 616518	1066	<i>F. chiloensis</i> f. <i>patagonica</i>	2 Lago Carrera 1A	H	Chile
PI 616519	1067	<i>F. chiloensis</i> f. <i>patagonica</i>	2 TAP 4C Elite #2	H	Chile
PI 602578	1193	<i>F. vesca</i> f. <i>alba</i>	Olympia (R. Clark)	H	Washington
PI 552247	956	<i>F. vesca</i> subsp. <i>americana</i>	WC44	H	New Hampshire
PI 637947	1817	<i>F. vesca</i> f. <i>bracteata</i>	OCJ-55	H	New Mexico
PI 616872	1614	<i>F. vesca</i> f. <i>semperflorens</i>	Everblooming vesca	H	Louisiana
PI 616610	1257	<i>F. vesca</i> subsp. <i>vesca</i>	Irkustk	H	Russia
PI 616932	1681	<i>F. virginiana</i> subsp. <i>glauca</i>	Sled Dog 1, North Pole	F	Alaska
PI 612496	1698	<i>F. virginiana</i> subsp. <i>glauca</i>	MN 8688	H	Alaska
PI 612501	1703	<i>F. virginiana</i> subsp. <i>glauca</i>	LH 30-4	H	Montana
PI 551642	279	<i>F. virginiana</i> subsp. <i>glauca</i>	Naples	H	Idaho
PI 551877	549	<i>F. virginiana</i> subsp. <i>glauca</i>	LH 20-1	H	Montana
PI 616601	1221	<i>F. virginiana</i> subsp. <i>platypetala</i>	Strawberry Mountain	H	Oregon
PI 616676	1366	<i>F. virginiana</i> subsp. <i>virginiana</i>	N-8	H	Quebec, Canada
PI 616667	1351	<i>F. virginiana</i> subsp. <i>virginiana</i>	Minnesota #32	F	Minnesota

²CFRA = Corvallis *Fragaria* number.

explorers likely brought them to foreign ports for food during their voyages because of this trait (Liston et al., 2014). In this study, *F. vesca* subsp. *vesca* f. *semperflorens* accessions from Europe and Kyrgyzstan, and *F. vesca* f. *alba* from Kentucky, Nova Scotia, Japan, and Hawaii were observed.

Darrow (1966) generalized that of the three North American octoploids, *F. ovalis* was often PF, *F. virginiana* was rarely PF, and *F. chiloensis* was SF. These broad statements have been assumptions that have influenced strawberry breeding decisions until recently. Staudt (1989) redefined North American strawberry taxonomy by dividing *F. ovalis* into *Fragaria virginiana* subsp. *glauca* and *F. virginiana* subsp. *platypetala*. Both in Hummer et al. (2016) and this study, PF clones were observed in each of the four subspecies of *F. virginiana* and, surprisingly, in each of the subspecies of *F. chiloensis* (Table 1). To have PF plants in the South American distribution of *F. chiloensis* subsp. *chiloensis* is of great interest. Original importations of these plants into Europe may have led to some of the earliest European PF *F. xananassa* cultivars. Mapping studies and molecular characterizations will be needed to validate this hypothesis.

The PF habit predominated in many genotypes of *F. virginiana* subsp. *glauca* which has historically been known for its drought tolerance, resistance to cold, and ability to flower multiple times, making select clones very useful for breeding efforts (Reed, 1966). Powers, a strawberry breeder at the USDA in Cheyenne, WY, obtained diverse types of the native Rocky Mountain strawberry, that he referred to as *F. ovalis*, and crossed and backcrossed it with *F. xananassa* cultivars to introgress many of the favorable traits observed in the wild germplasm (Powers, 1945, 1954). He concluded that multiple genes determined PF in strawberries and that some dominantly inherited genes had larger contributions toward producing PF progeny (Powers, 1945, 1954). The conclusions of Powers (1945, 1954) may have been confounded by inadvertently having multiple sources of the PF phenotype within his crossing experiment (Bringhurst et al., 1989). However, later

studies into the *F. virginiana* subsp. *glauca* Wasatch source of PF support the role that both major and minor effect genes have on flower habit (Gaston et al., 2013; Hancock et al., 2002; Perrotte et al., 2016a, 2016b).

Several taxa responded to the additional heat units available in 2016 with an increase in the number of PF clones (Fig. 1; Table 1). Three taxa in particular, *F. chiloensis* subsp. *chiloensis* f. *patagonica*, *F. virginiana* subsp. *virginiana*, and *F. xananassa*, had more PF individuals in 2016 than in 2015 (Table 1). Temperatures at the Corvallis, OR airport, for 2015 were generally similar to 2016, but differed in growing degree days during January, February, March, April, and May (Fig. 1; NOAA, 2017). During these months, temperatures were on average warmer in 2016 than in 2015 resulting in more growing degree days (Fig. 1). Seasonal cues, such as early spring warmth, positively affect PF in strawberry (Andrés and Coupland, 2012; Salinas et al., 2017).

Specific genotypes, particularly the 15 individuals that demonstrated the strong PF trait (Table 2), should be considered by *Fragaria* breeding programs for broadening the parental gene pools. Salinas et al. (2017) evaluated 19 *F. chiloensis* and *F. virginiana* accessions for PF during the validation of a diagnostic marker for the Wasatch source of PF. One of the *F. virginiana* subsp. *glauca* accessions that they evaluated was PI 612501 also known as CFRA 1703. In the present study, this accession exhibited PF during both 2015 and 2016; however, PI 612501 was only observed to be PF in two of the five environments in which it was evaluated (Salinas et al., 2017). Moreover, PI 612501 did not test positive for the *FaPFRU* locus, indicating that the marker developed in *F. xananassa* is not diagnostic of PF in *F. virginiana* or that PF in this accession may be controlled by different genes (Salinas et al., 2017). Breeders should be aware that the 15 individuals identified in the present study may exhibit different flowering responses depending on environmental conditions. Further research is needed to understand driving factors of PF and regulation in the different germplasm sources such as those observed in

this study. Presently, the Wasatch source is only commercially important for octoploids. This study suggests multiple sources of remontancy exist and could be implemented in commercial breeding programs.

Conclusion

While the SF habit appears in most strawberry species, the ability for clones to bloom perpetually is broadly found across many strawberry species and taxa of different ploidy levels. Although most representatives of a taxon may bloom seasonally, some genotypes can demonstrate the PF trait. Genetics and environment both affect flowering habit in strawberry genotypes. While diverse individuals of North and South American and some European taxa were amply represented, available samples of some Asian taxa were limited, therefore conclusions on their PF tendency could not be determined. This study evaluated flowering habit in existing representatives of taxa present in the NCGR collection. Additional representatives are needed to confirm PF tendencies in these taxa.

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Description of R Script

The input data file is structured with column 1 listing the groups and columns 2 and 3 listing the number of individuals with phenotype 1 and phenotype 2 for each group. The code creates two-by-two contingency tables for each combination of the groups, performs chi-square analysis, applies the N-1 correction, and writes an output table with the P-values from each test. The output table will have each group which was compared and the P-value from the chi-square test with N-1 correction. Warning messages will be produced regarding the chi-squared approximation. These messages are normal when performing the chi-squared test in R.

```
input_dat = read.table(choose.files(caption="Select input data"),header = TRUE)
setwd(choose.dir(caption="Select location to write output to"))
Group_names = as.character(input_dat[,1])
data = cbind.data.frame(input_dat[,2],input_dat[,3])
output = data.frame("Group_1"=as.character("Group_1"), "Group_2"=as.character("Group_2"),
"P-Value"=as.character("P-Value"),stringsAsFactors = FALSE)
Group1 = 1
Group2 = 2
while (Group1 < length(Group_names)){
  while (Group2 < length(Group_names)+1){
    conttable = rbind.data.frame(data[Group1,],data[Group2,])
    chisq = chisq.test(conttable,correct = FALSE)
    total = as.numeric(sum(conttable))
    pval = as.character(1-pchisq(chisq$statistic*((total-1)/total),1))
    newline = c(Group_names[Group1],Group_names[Group2],pval)
    output = rbind.data.frame(output,newline)
    Group2 = Group2 + 1
  }
  Group1 = Group1+1
  Group2 = Group1+1
}
write.table(output,file="output.txt",sep="\t",row.names = FALSE, col.names = FALSE)
```